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(54) Title: CONFORMATIONALLY CONSTRAINED BACKBONE CYCLIZED SOMATOSTATIN ANALOGS

(57) Abstract

Novel peptide analogs are disclosed. The novel peptides are conformationally constrained backbone cyclized somatostatin analogs. Methods for synthesizing the somatostatin analogs and for producing libraries of the somatostatin analogs are also disclosed. Furthermore, pharmaceutical compositions comprising somatostatin analogs, and methods of using such compositions in the treatment of endocrine disorders, neoplasms and metabolic disorders are also disclosed.

CONFORMATIONALLY CONSTRAINED BACKBONE
CYCLIZED SOMATOSTATIN ANALOGS

FIELD OF THE INVENTION

The present invention relates to conformationally constrained N^α backbone-cyclized somatostatin analogs cyclized via novel linkages, to processes for the preparation of these backbone cyclized peptide analogs, to methods for using these peptide analogs and to pharmaceutical compositions containing same.

BACKGROUND OF THE INVENTION

Somatostatin analogs

Somatostatin is a cyclic tetradecapeptide found both in the central nervous system and in peripheral tissues. It was originally isolated from mammalian hypothalamus and identified as an important inhibitor of growth hormone secretion from the anterior pituitary. Its multiple biological activities include inhibition of the secretion of glucagon and insulin from the pancreas, regulation of most gut hormones and regulation of the release of other neurotransmitters involved in motor activity and cognitive processes throughout the central nervous system (for review see Lamberts, Endocrine Rev., 9:427, 1988). Additionally, somatostatin and its analogs are potentially useful antiproliferative agents for the treatment of various types of tumors.

Natural somatostatin (also known as Somatotropin Release Inhibiting Factor, SRIF) of the following structure:

H-Ala^1-Gly^2-Cys^3-Lys^4-Asn^5-Phe^6-Phe^7-Trp^8-Lys^9-Thr^{10}-Phe^{11}-Thr^{12}-Ser^{13}-Cys^{14}-OH

was first isolated by Guillemin and colleagues (Bruzeau et al. Science, 179:78, 1973). It exerts its effect by interacting with a family of receptors. Recently five receptor subtypes, termed SSTR1-5, have been identified and cloned. In its natural form, somatostatin has limited use as a therapeutic agent since it exhibits two undesirable

properties: poor bioavailability and short duration of
action. For this reason, great efforts have been made during
the last two decades to find somatostatin analogs that will
have superiority in either potency, biostability, duration of
5 action or selectivity with regard to inhibition of the
release of growth hormone, insulin or glucagon.

Structure-activity relation studies, spectroscopic
techniques such as circular dichroism and nuclear magnetic
resonance, and molecular modeling approaches reveal the
10 following: the conformation of the cyclic part of natural
somatostatin is most likely to be an antiparallel β-sheet;
Phe^6 and Phe^{11} play an important role in stabilizing the
pharmacophore conformation through hydrophobic interactions
between the two aromatic rings; the four amino acids Phe^7-
15 Trp^9-Lys^9-Thr^{10} which are spread around the β-turn in the
antiparallel β-sheet are essential for the pharmacophore; and
$(D)Trp^8$ is preferable to $(L)Trp^8$ for the interactions with
somatostatin receptor subtypes 2 through 5.

Nevertheless, a hexapeptide somatostatin analog
20 containing these four amino acids anchored by a disulfide
bridge:

$$Cys-Phe^7-(D)Trp^8-Lys^9-Thr^{10}-Cys$$
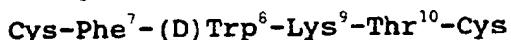
25 is almost inactive both *in vitro* and *in vivo*, although it has
the advantage of the covalent disulfide bridge which replaces
the Phe^6-Phe^{11} hydrophobic interactions in natural
somatostatin.

Four main approaches have been attempted in order to
30 increase the activity of this hexapeptide somatostatin
analog. (1) Replacing the disulfide bridge by a cyclization
which encourages a cis-amide bond, or by performing a second
cyclization to the molecule yielding a bicyclic analog. In
both cases the resultant analog has a reduced number of
35 conformational degrees of freedom. (2) Replacing the
original residues in the sequence Phe^7-$(D)Trp^8$-Lys^9-Thr^{10} with
other natural or non-natural amino acids, such as replacing

Phe[7] with Tyr[7] and Thr[10] with Val[10].   (3) Incorporating
additional functional groups from natural somatostatin with
the intention that these new elements will contribute to the
interaction with the receptor.   (4) Eliminating one of the
5 four amino acids Phe[7]-(D)Trp[8]-Lys[9]-Thr[10] with the assumption
that such analogs would be more selective.

The somatostatin analog, MK-678:

cyclo(N-Me-Ala[6]-Tyr[7]-(D)Trp[8]-Lys[9]-Val[10]-Phe)

10

is an example of a highly potent somatostatin analog designed
using the first three approaches above (Veber, et al., Life
Science, 34:371, 1984).   In this hexapeptide analog, a cis-
amide bond is located between N-Me-Ala and Phe[11], Tyr[7] and
15 Val[10] replace Phe[7] and Thr[10] respectively, and Phe[11] is
incorporated from natural somatostatin.

Another group of somatostatin analogs (U.S. patents
4,310,518 and 4,235,886) includes Octreotide:

20 H-(D)Phe[5]-Cys-Phe[7]-(D)Trp[8]-Lys[9]-Thr[10]-Cys-Thr[12]-CH$_2$OH
       |_____|

the only approved somatostatin analog currently available.
It was developed using the third approach described above.
25 Here, (D)Phe[5] and the reduced C-terminal Thr[12]-CH$_2$OH are
assumed to occupy some of the conformational space available
to the natural Phe[6] and Thr[12], respectively.

The compound TT-232:

30
   H-(D)Phe-Cys-Phe[7]-(D)Trp[8]-Lys[9]-Cys-Thr-NH$_2$
         |_____|

is closely related to Octreotide and is an example of
35 implementing the fourth approach described above.   The lack
of Thr[10] is probably responsible for its high functional
selectivity in terms of antitumor activity.

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These examples of highly potent somatostatin analogs
suggest that the phenylalanines in positions 6 and 11 not
only play an important role in stabilizing the pharmaconhore
conformation but also have a functional role in the
5 interaction with the receptor. It is still an open question
whether one phenylalanine (either Phe^6 or Phe^{11}) is sufficient
for the interaction with the receptor or whether both are
needed.

It is now known that the somatostatin receptors
10 constitute a family of five different receptor subtypes (Bell
and Reisine, _Trends Neurosci._, _16_, 34-38, 1993), which may be
distinguished on the basis of their tissue specificity and/or
biological activity. Somatostatin analogs known in the art
may not provide sufficient selectivity or receptor subtype
15 selectivity, particularly as anti-neoplastic agents (Reubi
and Laissue, _TIPS_, _16_, 110-115, 1995).

Symptoms associated with metastatic carcinoid tumors
(flushing and diarrhea) and vasoactive intestinal peptide
(VIP) secreting adenomas (watery diarrhea) are treated with
20 somatostatin analogs. Somatostatin has been also approved
for the treatment of severe gastrointestinal hemorrhages.
Somatostatin may also be useful in the palliative treatment
of other hormone-secreting tumors (e.g., pancreatic islet-
cell tumors and acromegaly) and hormone dependent tumors
25 (e.g., chondrosarcoma and osteosarcoma) due to its
antisecretory activity.

Peptidomimetics

As a result of major advances in organic chemistry and
30 in molecular biology, many bioactive peptides can now be
prepared in quantities sufficient for pharmacological and
clinical utilities. Thus in the last few years new methods
have been established for the treatment and therapy of
illnesses in which peptides have been implicated. However,
35 the use of peptides as drugs is limited by the following
factors: a) their low metabolic stability towards proteolysis
in the gastrointestinal tract and in serum; b) their poor

absorption after oral ingestion, in particular due to their
relatively high molecular mass or the lack of specific
transport systems or both; c) their rapid excretion through
the liver and kidneys; and d) their undesired side effects in
5 non-target organ systems, since peptide receptors can be
widely distributed in an organism.

Moreover, with few exceptions, native peptides of small
to medium size (less than 30 amino acids) exist unordered in
dilute aqueous solution in a multitude of conformations in
10 dynamic equilibrium which may lead to lack of receptor
selectivity, metabolic susceptibilities and hamper attempts
to determine the biologically active conformation. If a
peptide has the biologically active conformation per se,
i.e., receptor-bound conformation, then an increased affinity
15 toward the receptor is expected, since the decrease in
entropy on binding is less than that on the binding of a
flexible peptide. It is therefore important to strive for
and develop ordered, uniform and biologically active
peptides.

20 In recent years, intensive efforts have been made to
develop peptidomimetics or peptide analogs that display more
favorable pharmacological properties than their prototype
native peptides. The native peptide itself, the
pharmacological properties of which have been optimized,
25 generally serves as a lead for the development of these
peptidomimetics. However, a major problem in the development
of such agents lies in determining the active region of a
biologically active peptide. For instance, frequently only a
small number of amino acids (usually four to eight) are
30 responsible for the recognition of a peptide ligand by a
receptor. Once this biologically active site is determined a
lead structure for development of peptidomimetic can be
optimized, for example by structure-activity relationship
studies.

35 As used herein, a "peptidomimetic" is a compound that,
as a ligand of a receptor, can imitate (agonist) or block
(antagonist) the biological effect of a peptide at the

receptor level. The following factors should be considered
to achieve the best possible agonist peptidomimetic a)
metabolic stability, b) good bioavailability, c) high
receptor affinity and receptor selectivity, and d) minimal
5 side effects.

A generally applicable and successful method recently
has been the development of conformationally restricted
peptidomimetics that imitate the receptor-bound conformation
of the endogenous peptide ligands as closely as possible
10 (Rizo and Gierasch, Ann. Rev. Biochem., 61:387, 1992).
Investigations of these types of analogs show them to have
increased resistance toward proteases, that is, an increase
in metabolic stability, as well as increased selectivity and
thereby fewer side effects (Veber and Friedinger, Trends
15 Neurosci., p. 392, 1985).

Once these peptidomimetic compounds with rigid
conformations are produced, the most active structures are
selected by studying the structure-activity relationships.
Such conformational constraints can involve local
20 modifications of structure or global conformational
restraints (for review see Giannis and Kolter, Angew. Chem.
Int. Ed. Engl. 32:1244, 1993).

Conformationally constrained peptide analogs

25   Bridging between two neighboring amino acids in a
peptide leads to a local conformational modification, the
flexibility of which is limited in comparison with that of
regular dipeptides. Some possibilities for forming such
bridges include incorporation of lactams and piperazinones.
30 γ-Lactams and δ-lactams have been designed to some extent as
"turn mimetics"; in several cases the incorporation of such
structures into peptides leads to biologically active
compounds.

Global restrictions in the conformation of a peptide are
35 possible by limiting the flexibility of the peptide strand
through cyclization (Hruby et al., Biochem. J., 268:249,
1990). Not only does cyclization of bioactive peptides

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improve their metabolic stability and receptor selectivity,
cyclization also imposes constraints that enhance
conformational homogeneity and facilitates conformational
analysis.   The common modes of cyclization are the same found
5 in naturally occurring cyclic peptides.   These include side
chain to side chain cyclization or side chain to end-group
cyclization.   For this purpose, amino acid side chains that
are not involved in receptor recognition are connected
together or to the peptide backbone.   Another common
10 cyclization is the end-to-end cyclization.

The main limitations to these classical modes of
cyclization are that they require substitution of amino acid
side chains in order to achieve cyclization.

Another conceptual approach to the conformational
15 constraint of peptides was introduced by Gilon, et al., (Bio-
polymers 31:745, 1991) who proposed backbone to backbone
cyclization of peptides.   The theoretical advantages of this
strategy include the ability to effect cyclization via the
carbons or nitrogens of the peptide backbone without
20 interfering with side chains that may be crucial for
interaction with the specific receptor of a given peptide.
While the concept was envisaged as being applicable to any
linear peptide of interest, in point of fact the limiting
factor in the proposed scheme was the availability of
25 suitable building units that must be used to replace the
amino acids that are to be linked via bridging groups.   The
actual reduction to practice of this concept of backbone
cyclization was prevented by the inability to devise any
practical method of preparing building units of amino acids
30 other than glycine (Gilon et al., J. Org. Chem, 587:5687,
1992).

In Gilon, EPO Application No. 564,739 A2; and J. Org.
Chem., 57:5687, 1992, two basic approaches to the synthesis
of building units are described.   The first starts with the
35 reaction of a diamine with a bromo acid.   Selective
protection of the ω amine and further elaboration of
protecting groups provides a building unit, suitable for Boc

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chemistry peptide synthesis. The second approach starts with
selective protection of a diamine and reaction of the product
with chloroacetic acid to provide the protected glycine
derivative, suitable for Fmoc peptide synthesis.

5   Further modes of utilizing the concept of backbone
cyclized peptide analogs are disclosed in WO95/33765 which
also provides novel methods of synthesizing building units
other than glycine. Among the families of peptide analogs
disclosed in WO95/33765 are various somatostatin analogs.

10 None of the families of analogs disclosed in that application
is shown to have unique attributes or unexpected advantages
in terms of the binding to receptor subtypes.

Libraries of backbone cyclized peptide analogs

15   As mentioned above linear peptides suffer from several
serious drawbacks as potential drugs, inasmuch as they are
notoriously unstable in vivo, often lack high affinity of
binding to their receptor, frequently lack selectively to one
kind of receptor, and generally have poor oral

20 bioavailability. In efforts to overcome such problems, it is
also possible to utilize the methodologies developed in
connection with synthetic peptide libraries to generate
collections of cyclic peptides, novel biopolymers and even
novel branched oligomeric compounds (reviewed by Zuckermann,

25 Current Opinion in Structural Biology 3, 580-584, 1993).

      The generation of libraries of cyclic peptides requires
that in addition to any previously stated considerations, the
cyclization reaction be performed in a high yield and with a
minimum of additional manipulations. Unfortunately,

30 classical cyclization reactions are highly sequence dependent
in terms of the expected yields, making the uniform
cyclization of a peptide mixture unreliable.

      Recent advances in the cyclization of peptides directly
on the solid support have improved the synthetic procedure,

35 and even allowed the automation of cyclization reactions
based on known cyclization schemes. In the past,
cyclizations were typically performed in solution under

conditions of high dilution. Polymer-supported cyclizations

can both avoid potential side reactions such as

oligomerization and facilitate product purification. For

example, on-resin cyclization methods have recently been used

5 to prepare cyclopeptides with bridges formed of thioethers,

disulfides, or lactams between two side chains, lactam

between the amino terminus and a side chain, and lactams

between the amino and carboxy termini (reviewed by

Zuckermann, Current Opinion in Structural Biology 3, ibid).

10   The use of resin-bound cyclic peptides and free cyclic

peptides in combinatorial libraries is disclosed in WO

92/00091. However, these cyclic peptides do not contain any

conformationally constraining element, and in cases where

cyclization is achieved, these peptides may still adopt a

15 number of conformations and suffer many of the same

shortcomings as linear peptides.

Cyclic semi-random peptide libraries, disclosed in WO

95/01800, are exclusively cyclic penta- and hexa-peptide

libraries containing one or more randomized amino acids and a

20 conformationally constraining element in the form of an amino

acid residue such as proline which fixes the beta turn angles

of the adjacent amino acid residues. The advantages of such

conformationally constraining elements is stressed by the

inventors of this approach. However, inclusion of such

25 elements via incorporation of a particular amino acid residue

into the peptide sequence may have detrimental effects on

those residues required for receptor recognition or other

biological activity. Furthermore, in that disclosure (WO

95/01800), the cyclization reaction is merely another

30 coupling reaction in which the terminal amino group of the

linear peptide is coupled to the terminal carboxy group of

the peptide.

35

SUMMARY OF THE INVENTION

According to the present invention, novel peptidomimetic compounds, which are characterized in that they incorporate novel building units with bridging groups attached to the
5 alpha nitrogens of alpha amino acids; have now been generated.

The most striking advantages of this approach are:
1) The method enables cyclization of the peptide sequence without compromising any of the side chains of the peptide
10 thereby decreasing the chances of sacrificing functional groups essential for biological recognition and function.
2) The method allows optimization of the peptide conformation by allowing permutation of the bridge length, direction, and bond type (e.g., amide, disulfide, thioether, thioester,
15 etc.) and position of the bond in the ring. 3) When applied to cyclization of linear peptides of known activity, the bridge can be designed in such a way as to minimize interaction with the active region of the peptide and its cognate receptor. This decreases the chances of the
20 cyclization arm interfering with recognition and function, and also creates a site suitable for attachment of tags such as radioactive tracers, cytotoxic drugs, light capturing substances, or any other desired label.

The newly generated libraries, disclosed according to
25 the present invention, now enable varying conformation as well as level of flexibility (constraint) in order to find the optimal backbone conformation of the peptide in performing its role as an agonist or antagonist. This is accomplished by varying both the position of the bridgeheads
30 (i.e., the positions in the linear sequence of residues that are to be cyclized), as well as varying the length, the direction and the bond type of the bridge between these units.

It is another object of the present invention to provide
35 backbone cyclized somatostatin analogs that comprise peptide sequences which contain one nitrogen atom of the peptide backbone connected to a bridging group, as described below.

- 10 -

In the present invention, one or more pairs of the building units is joined together to form a cyclic structure. Thus, according to one aspect of the present invention, backbone cyclized somatostatin analogs are provided that have the

5 general Formula (I):

Q-(AA)$_a$-N-CH(R^1)-CO-(AA)$_b$-N-CH(R^2)-CO-(AA)$_c$-E
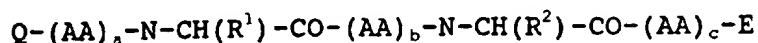
Formula (I)

10

wherein: a - c each independently designates an integer from 1 to 8 or zero; (AA) designates an amino acid residue wherein the amino acid residues in each chain may be the same or different; Q represents H or an acyl group; E represents a

15 hydroxyl group, a carboxyl protecting group or an amino group, or the terminal carboxyl group can be reduced to CH$_2$-OH; R^1 and R^2 each designates an amino acid side-chain optionally bound with a specific protecting group; and the line designates a bridging group of the Formula:

20 (i) -X-M-Y-W-Z- ; or (ii) -X-M-Z-wherein: M and W are independently selected from the group consisting of amide, thioether, thioester and disulfide; and X, Y and Z are each independently selected from the group consisting of alkylene, substituted alkylene, arylene, homo- or hetero- cycloalkylene

25 and substituted cycloalkylene.

In certain preferred embodiments, the terminal carboxyl group of Formula (I) is replaced by a carboxy terminal amide or is reduced to a CH$_2$OH group.

Another embodiment of the present invention involves

30 N-backbone to side chain cyclized somatostatin analogs of the general formula (II):

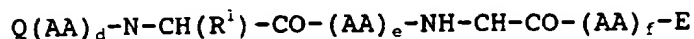Q(AA)$_d$-N-CH(R^1)-CO-(AA)$_e$-NH-CH-CO-(AA)$_f$-E

35

Formula (II)

wherein: d - f each independently designates an integer from
1 to 8 or zero; (AA) designates an amino acid residue wherein
the amino acid residues in each chain may be the same or
different; Q represents H or an acyl group; E represents a
5   hydroxyl group, a carboxyl protecting group or an amino
group, or the terminal carboxyl group can be reduced to CH_2-
OH; R^1 designates an amino acid side-chain optionally bound
with a specific protecting group; and the line designates a
bridging group of the Formula: (i) -X-M-Y-W-Z- ; or
10 (ii) -X-M-Z- wherein M and W are independently selected from
the group consisting of amide, thioether, thioester and
disulfide; and X, Y and Z are each independently selected
from the group consisting of alkylene, substituted alkylene,
arylene, homo- or hetero- cycloalkylene and substituted
15 cycloalkylene.

A preferred embodiment of the present invention involves
the backbone cyclized somatostatin analog of Formulae I or II
wherein the line designates a bridging group of the Formula
$-(CH_2)_x-M-(CH_2)_y-$; M is selected from the group consisting of
20 amide, thioether, thioester and disulfide; and x and y each
independently designates an integer from 1 to 10.

Further preferred are backbone cyclized somatostatin
analogs of the Formula I or II wherein R^1 and R^2 are other
than H, such as CH_3, $(CH_3)_2CH-$, $(CH_3)_2CHCH_2-$, $CH_3CH_2CH(CH_3)-$,
25 $CH_3S(CH_2)_2-$, $HOCH_2-$, $CH_3CH(OH)-$, $HSCH_2-$, $NH_2C(=O)CH_2-$,
$NH_2C(=O)(CH_2)_2-$, $NH_2(CH_2)_3-$, $HOC(=O)CH_2-$, $HOC(=O)(CH_2)_2-$,
$NH_2(CH_2)_4-$, $C(NH_2)_2 NH(CH_2)_3-$, HO-phenyl-CH_2-, benzyl,
methylindole, and methylimidazole.

Another preferred aspect of the present invention is
30 directed to backbone cyclization to generate novel
somatostatin analogs linked between positions 6 and 11,
leaving the phenylalanine side chains untouched. This
conformational stabilization is much more rigid than the Phe[6],
Phe[11] hydrophobic interaction in natural somatostatin and is
35 more stable to reduction/oxidation reactions than the Cys-Cys
disulfide bridge. In other words, for the first time a

stable covalent bridge can be achieved while either one or
both of the original Phe[6] and Phe[11] are retained.

Moreover, backbone cyclizations can also be used to
anchor the β-turn, not only in positions 6 and 11 but also
inside the active reaction of Phe[7]-(D)Trp[8]-Lys[9]-Thr[10], yielding
either a monocyclic analog with a preferable conformation or
a very rigid bicyclic analog. Here again, the side chains of
the pharmacologically active amino acids remain untouched and
the only change is in limiting the conformational space.

As used herein and in the claims in the following more
preferred backbone cyclized peptide analogs, the superscript
numbers following the amino acids refer to their position
numbers in the native Somatostatin.

A more preferred backbone cyclized somatostatin analog
is the Formula (Va):

$$R^5-NR^6-R^7-(D)Trp-Lys-R^{10}-NR^{11}-R^{12}-X$$
$$|\_\_\_(CH_2)_m-Y^2-(CH_2)_n\_\_\_|$$
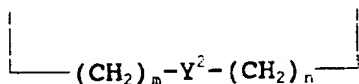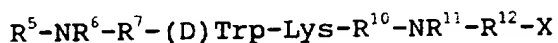
Formula (Va)

a most preferred analog having Formula (Vb):

$$H-NR^6-R^7-(D)Trp-Lys-R^{10}-NR^{11}-Thr-X$$
$$|\_\_\_(CH_2)_m-Y^2-(CH_2)_n\_\_\_|$$
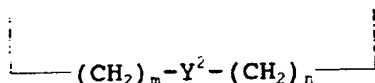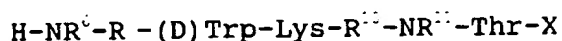
Formula (Vb)

wherein m and n are 1 to 5; X designates a carboxy terminal
amide or alcohol; R^5 is absent or is Gly, (D)- or (L)-Ala,
(D)- or (L)-Phe, Nal and β-Asp(Ind); R^6 and R^{11} are
independently Gly or (D)- or (L)-Phe; R^7 is Phe or Tyr; R^{10} is
absent or is Gly, Abu, Thr or Val; R^{12} is absent or is Val,
Thr or Nal, and Y^2 is selected from the group consisting of
amide, thioether, thioester and disulfide. In these
monocyclic somatostatin analogs, a backbone cyclization
replaces the Cys[6]-Cys[11] disulfide bridge, leaving the

phenylalanine side chains as in the natural somatostatin.
Still more preferred is the analog wherein Phe^7 is replaced
with Tyr^7 and Thr^{10} is replaced with Val^{10}.

Other preferred monocyclic analogs that anchor the
molecule in positions inside the active region rather than in
positions 6 and 11 are formulae VI (a and b) and VII (a-c):
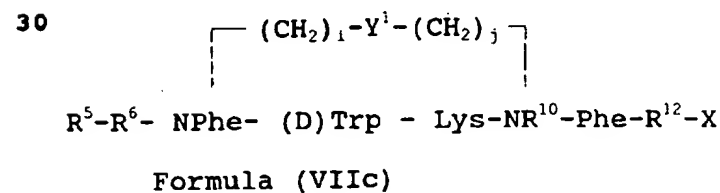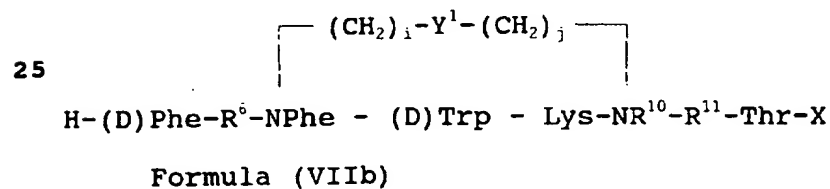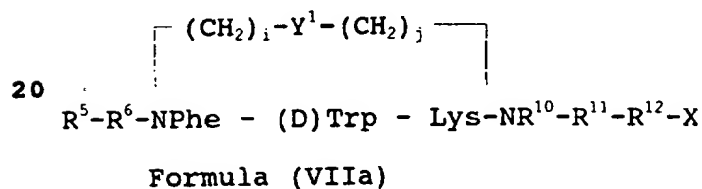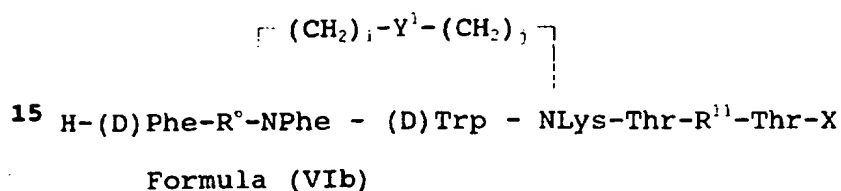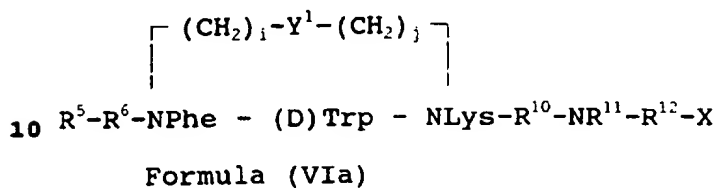
$$\overbrace{}^{(CH_2)_i - Y^1 - (CH_2)_j}$$
$$R^5 - R^6 - NPhe - (D)Trp - NLys - R^{10} - NR^{11} - R^{12} - X$$

Formula (VIa)

$$\overbrace{}^{(CH_2)_i - Y^1 - (CH_2)_j}$$
$$H - (D)Phe - R^6 - NPhe - (D)Trp - NLys - Thr - R^{11} - Thr - X$$

Formula (VIb)

$$\overbrace{}^{(CH_2)_i - Y^1 - (CH_2)_j}$$
$$R^5 - R^6 - NPhe - (D)Trp - Lys - NR^{10} - R^{11} - R^{12} - X$$

Formula (VIIa)

$$\overbrace{}^{(CH_2)_i - Y^1 - (CH_2)_j}$$
$$H - (D)Phe - R^6 - NPhe - (D)Trp - Lys - NR^{10} - R^{11} - Thr - X$$

Formula (VIIb)

$$\overbrace{}^{(CH_2)_i - Y^1 - (CH_2)_j}$$
$$R^5 - R^6 - NPhe - (D)Trp - Lys - NR^{10} - Phe - R^{12} - X$$

Formula (VIIc)

wherein i and j are independently 1 to 5; X designates a carboxy terminal amide or alcohol; R^5 is absent or is (D) - or (L)-Phe, Nal, or β-Asp(Ind); R^6 is (D) or (L) -Phe; R^{10} is absent or is Gly, Abu or Thr; and R^{11} is (D)- or (L)-Phe; R^{12}

5 is absent or is Thr or Nal, and Y^1 is selected from the group consisting of amide, thioether, thioester and disulfide.

Still other preferred analogs incorporate backbone cyclization in positions 6 and 11 as in Formula V, together with the backbone cyclizations as in Formula VI and VII,

10 yielding rigid bicyclic analogs.

Other more preferred bicyclic analogs differ from Formulae V-VII by the replacement of the amino acids at positions 6 and 11 by cysteines which form a disulfide bond, leaving only one backbone cyclization in the Formulae VIII(a
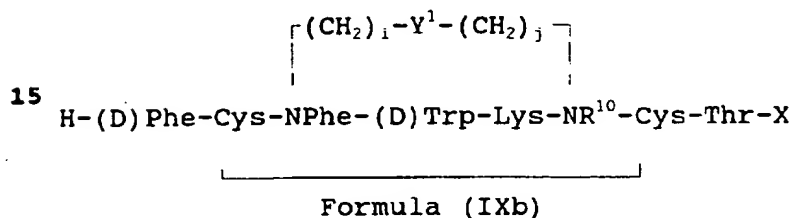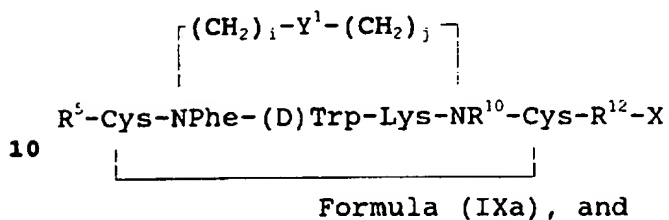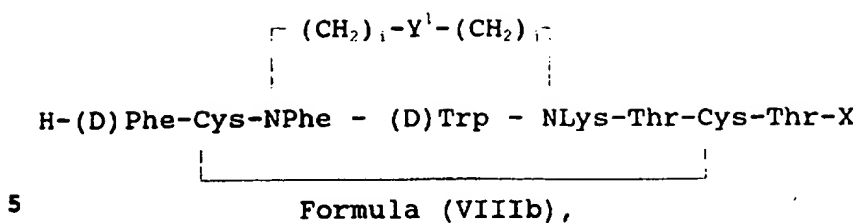
15 and b) and IX (a and b):

$$\overline{} (CH_2)_i - Y^1 - (CH_2)_j \overline{}$$

20 R^5 - Cys- NPhe - (D)Trp - NLys-R^{10}-Cys-R^{12}-X

Formula (VIIIa),

25

30

35

$$\overbrace{\quad-(CH_2)_i-Y^1-(CH_2)_i\quad}$$

H-(D)Phe-Cys-NPhe - (D)Trp - NLys-Thr-Cys-Thr-X

Formula (VIIIb),

$$\overbrace{(CH_2)_i-Y^1-(CH_2)_j}$$

R^5-Cys-NPhe-(D)Trp-Lys-NR^{10}-Cys-R^{12}-X

Formula (IXa), and

$$\overbrace{(CH_2)_i-Y^1-(CH_2)_j}$$

H-(D)Phe-Cys-NPhe-(D)Trp-Lys-NR^{10}-Cys-Thr-X

Formula (IXb)

wherein i and j are independently 1 to 5; X designates a carboxy terminal amide or alcohol; R^5 is absent or is (D)- or (L) -Phe, Nal, or β-Asp (Ind); R^6 and R^{11} are independently Gly or Phe; R^{10} is absent or is Gly, Abu or Thr; R^{12} is absent or is Thr or Nal; and Y^1 is selected from the group consisting of amide, thioether, thioester and disulfide.

The most preferred embodiments of the invention are currently:

$$(CH_2)_2-CO-NH-(CH_3)_3\ \overline{\qquad\qquad}$$

N-Phe-Tyr-(D)Trp-Lys-Val-NPhe-Thr-X

(i.e., cyclo [NPhe-Tyr-(D)Trp-Lys-Val-NPhe]-Thr-X:
denoted PTR 3046)

and

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$(CH_2)\,\text{-CO-HN-}(CH_3)_3$ ⎯⎯⎯⎯⎯⎤
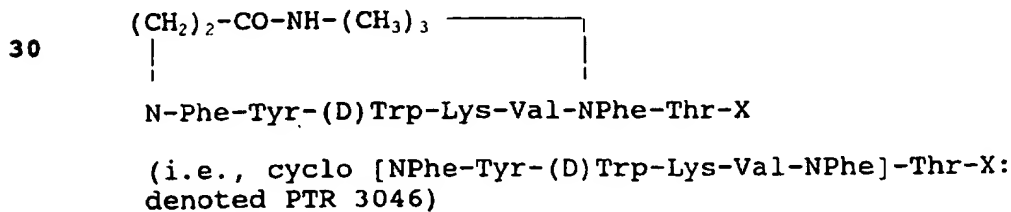
N-Phe-Phe-(D)Trp-Lys-Thr-NPhe-Val-X

(i.e., cyclo [NPhe-Phe-(D)Trp-Lys-Thr-NPhe]-Val-X:
denoted PTR 3040)

wherein X denotes a carboxy terminal acid, amide, ester or
alcohol. These two analogs have been shown to possess
unexpectedly useful properties due to their selectivity to
particular somatostatin receptor subtypes.

The more preferred monocyclic somatostatin analogs may
also be prepared as libraries of active analogs, that are
particularly useful to screen for the optimal conformers.

Yet more preferred somatostatin analogs according to the
present invention include compositions of the formulae X
through XIV:

⎯⎯ $(CH_2)_i$-Y^1-$(CH_2)_j$ ⎯⎯⎤

R^5-NR^6-R^7-(D)Trp-Lys-R^{10}-NR^{11}-R^{12}-X
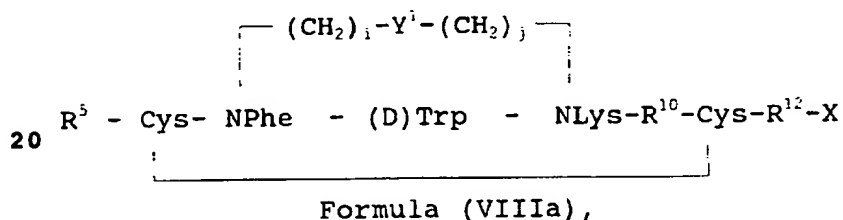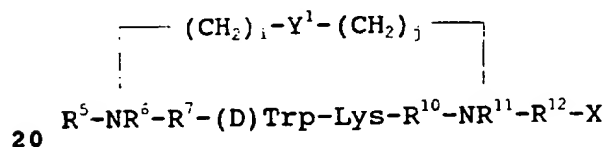
Formula X

wherein i and j are independently 1 to 5; X designates a
carboxy terminal amide or alcohol; R^5 is (D)Phe or 2-Nal; R^6
is Phe, Gly or Ala; R^7 is Tyr or pClPhe; R^{10} is Thr, Val, Ser
or Abu; R^{11} is Phe, Gly or Ala; R^{12} is Thr, Val, 2-Nal or (D)2-
Nal, and Y^1 is selected from the group consisting of amide,
thioether, thioester and disulfide.

Formula XI

⎡⎯⎯ $(CH_2)_i$-Y^1-$(CH_2)_j$ ⎯⎯⎤

NR^5-R^6-R^7-(D)Trp-Lys-R^{10}-NR^{11}-R^{12}-X

wherein i and j are independently 1 to 5; X designates a
carboxy terminal amide or alcohol; R^5 is (D)Phe or (L)Phe, Ala

or Lys; R^6 is absent or is Phe; R^7 is Tyr or Phe; R^{10} is absent or Thr, Val, Ser or Abu; R^{11} is Phe, Gly or Ala; R^{12} is Trp, Thr, Val, 2-Nal or (D)2-Nal, and Y^1 is selected from the group consisting of amide, thioether, thioester and disulfide.

Formula XII

$$\overline{(CH_2)_i-Y^1-(CH_2)_j}$$
$$R^{12}-NR^{11}-R^{10}-(D)Lys-Trp-R^7-NR^6-R^5-X$$

wherein i and j are independently 1 to 5; X designates a carboxy terminal amide or alcohol; R^5 Phe, (L)2-Nal or (D)2-Nal; R^6 is Phe, Gly or Ala; R^7 is (D)Phe, pCl(D)Phe, pNH$_2$Phe or (D)Tyr; R^{10} is (D)Thr, (D)Val (D)Ala, (D)Leu or (D)Glu; R^{11} is Phe, Gly or Ala; R^{12} is absent or is Thr or Val; and Y^1 is selected from the group consisting of amide, thioether, thioester and disulfide.

Formula XIII

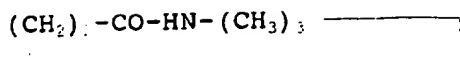$$\overline{(CH_2)_i-Y^1-(CH_2)_j}$$
$$R^5-NR^6-R^7-R^8-Lys-Gly-NR^{11}-R^{12}-X$$
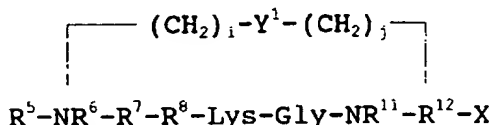
wherein i and j are independently 1 to 5; X designates a carboxy terminal amide or alcohol; R^5 is absent or (D)Phe or 2-Nal; R^6 is Phe, Gly or Ala; R^7 is (D)Phe, pCl(D)Phe, pNH$_2$Phe or (D)Tyr; R^8 is (D) or (L)Trp; R^{10} is (D)Thr, (D)Val, (D)Ala, (D)Leu or (D)Glu; R^{11} is Phe, Gly or Ala; R^{12} is Thr, Val, Ala, β-Ala, (L)2-Nal or (D)2-Nal; and Y^1 is selected from the group consisting of amide, thioether, thioester and disulfide.

Formula XIV

$$\overline{(CH_2)_i-Y^1-(CH_2)_j}$$
$$R^1-Gly-NR^3-R^4-R^5-Phe-NR^-DTrp-Lys-X$$
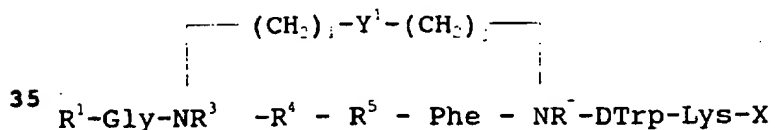
wherein i and j are independently 1 to 5; X designates a carboxy terminal amide or alcohol; R^1 is Ala or (D)2-Nal; R^3 is Phe, Gly, Ala or Lys; R^4 is Lys or Arg; R^5 is (L)Asn or (D)Asn; R^7 is Phe, Gly, Ala or Lys, and Y^1 is selected from the group consisting of amide, thioether, thioester and disulfide.

Another aspect of the present invention is a method for the preparation of cyclic peptides of the general Formula (I):
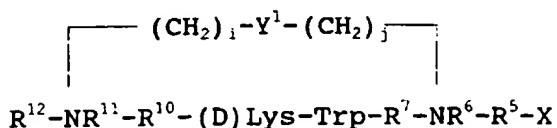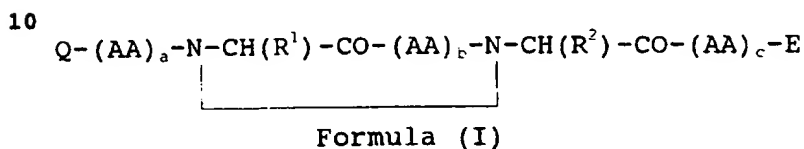
$$Q-(AA)_a-N-CH(R^1)-CO-(AA)_b-N-CH(R^2)-CO-(AA)_c-E$$

Formula (I)

wherein: a-c each independently designates an integer from 1 to 8 or zero; (AA) designates an amino acid residue wherein the amino acid residues in each chain may be the same or different; Q represents H or an acyl group; E represents a hydroxyl group, a carboxyl protecting group or an amino group, or the terminal carboxyl group can be reduced to CH_2-OH; R^1 to R^4 each designates an amino acid side-chain optionally bound with a specific protecting group; and the lines designate a bridging group of the Formula:
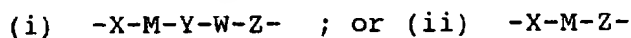
(i)   -X-M-Y-W-Z-   ; or (ii)   -X-M-Z-

wherein M and W are independently selected from the group consisting of amide, thioether, thioester and disulfide; and X, Y and Z are each independently selected from the group consisting of alkylene, substituted alkylene, arylene, homo- or hetero-cycloalkylene and substituted cycloalkylene.

This method comprises the steps of incorporating at least one N^α-ω-functionalized derivative of amino acids of Formula (III):
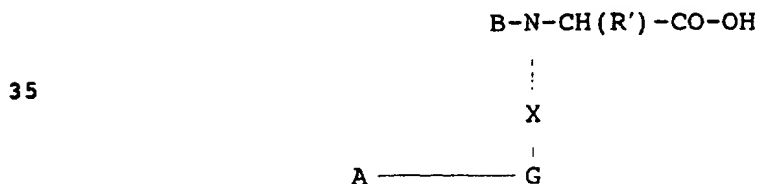
$$B-N-CH(R')-CO-OH$$

X

A ——————— G

- 19 -

Formula (III)

wherein X is a spacer group selected from the group
consisting of alkylene, substituted alkylene, arylene,
5 cycloalkylene and substituted cycloalkylene; R^1 is an amino
acid side chain, optionally bound with a specific protecting
group; B is a protecting group selected from the group
consisting of alkyloxy, substituted alkyloxy, or aryl
carbonyls; and G is a functional group selected from the
10 group consisting of amines, thiols, alcohols, carboxylic
acids and esters, aldehydes, alcohols and alkyl halides; and
A is a specific protecting group of G; into a peptide
sequence and subsequently selectively cyclizing the
functional group with one of the side chains of the amino
15 acids in said peptide sequence or with another ω-
functionalized amino acid derivative.

Preferred building units are the co-functionalized amino
acid derivatives wherein X is alkylene; G is a thiol group,
an amine group or a carboxyl group; R is phenyl, methyl or
20 isobutyl; with the proviso that when G is an amine group, R
is other than H.

Further preferred are ω-functionalized amino acid
derivatives wherein R is protected with a specific protecting
group.
25   More preferred are ω-functionalized amino acid
derivatives of Formula III, wherein G is an amino group, a
carboxyl group, or a thiol group:

```
    B-N-CH(R')-CO-OH        B-N-CH(R')-CO-OH            B-N-CH(R')-CO-OH
       | |                      |                            |
30     X                       X                            X
       |                       |                            |
       H                       CO        and                S
```
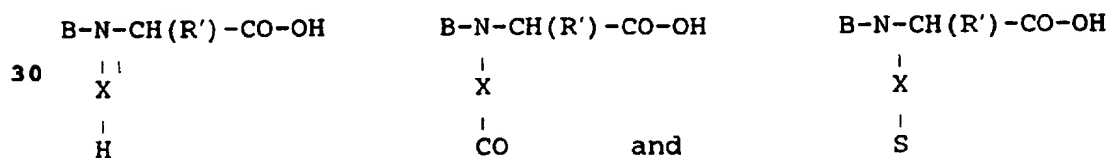
wherein X, R, A and B are as defined above.

35   A further aspect of this invention is to provide methods
for the preparation of novel backbone cyclic somatostatin
analogs, comprising the steps of incorporating at least one

N°-ω-functionalized derivative of an amino acid into a peptide
sequence and subsequently selectively cyclizing the
functional group with one of the side chains of the amino
acids in said peptide sequence, or with another ω-
5 functionalized amino acid derivative. Backbone cyclized
analogs of the present invention may be used as
pharmaceutical compositions and for methods for the treatment
of disorders including: post-surgical pain, all types of
inflammation, in particular pancreatitis, cancers, endocrine
10 disorders and gastrointestinal disorders.

     Therefore, further objects of the present invention are
directed to pharmaceutical compositions comprising
pharmacologically active backbone cyclized peptide agonists
and antagonists prepared according to the methods disclosed
15 herein and a pharmaceutically acceptable carrier or diluent;
and methods for the treatment of inflammation, cancer or
endocrine disorders and gastrointestinal disorders therewith.

BRIEF DESCRIPTION OF THE DRAWINGS

20   Figure 1 is a graph showing the inhibition of
somatostatin (SRIF-14) binding to the different SSTR
subtypes, as a function of the concentration of the backbone
cyclic somatostatin analog PTR 3046.

25   Figure 2 is a graph showing the inhibition of
somatostatin (SRIF-14) binding to the somatostatin receptors
on mouse pituitary AtT20 cell line as a function of the
concentration of the backbone cyclic somatostatin analogs PTR
3046 and PTR 3040.
30

     Figure 3 is a graphical comparison of the effects of
Octreotide and the backbone cyclic somatostatin analog PTR
3046 on growth hormone release in rats.

35 ·

Figure 4 is a graphical comparison of the effects of Octreotide and the backbone cyclic somatostatin analog PTR 3046 on insulin release in rats.

5   Figure 5 is a graphical comparison of the effects of Octreotide and the backbone cyclic somatostatin analog PTR 3046 on pancreatic exocrine release following bombesin induction.

10   Figure 6 is a graphical comparison of the antiproliferative effects of PTR 3046 and Octreotide on MiaPaca-2 cells.

15   **DETAILED DESCRIPTION OF THE INVENTION**
The compounds herein described may have asymmetric centers. All chiral, diastereomeric, and racemic forms are included in the present invention. Many geometric isomers of olefins and the like can also be present in the compounds
20 described herein, and all such stable isomers are contemplated in the present invention.
By "stable compound" or "stable structure" is meant herein a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction
25 mixture, and formulation into an efficacious therapeutic agent.
As used herein and in the claims, "alkyl" or "alkylenyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having one to ten
30 carbon atoms; "alkenyl" is intended to include hydrocarbon chains of either a straight or branched configuration having two to ten carbon atoms and one or more unsaturated carbon-carbon bonds which may occur in any stable point along the chain, such as ethenyl, propenyl, and the like; and "alkynyl"
35 is intended to include hydrocarbon chains of either a straight or branched configuration having from two to ten carbon atoms and one or more triple carbon-carbon bonds which

may occur in any stable point along the chain, such as
ethynyl, propynyl, and the like.

As used herein and in the claims, "aryl" is intended to
mean any stable 5- to 7-membered monocyclic or bicyclic or 7-
5 to 14-membered bicyclic or tricyclic carbon ring, any of
which may be saturated, partially unsaturated or aromatic,
for example, phenyl, naphthyl, indanyl, or tetrahydronaphthyl
tetralin, etc.

As used herein and in the claims, "alkyl halide" is
10 intended to include both branched and straight-chain
saturated aliphatic hydrocarbon groups having the one to ten
carbon atoms, wherein 1 to 3 hydrogen atoms have been
replaced by a halogen atom such as Cl, F, Br, and I.

As used herein and in the claims, the phrase
15 "therapeutically effective amount" means that amount of novel
backbone cyclized peptide analog or composition comprising
same to administer to a host to achieve the desired results
for the indications described herein, such as but not limited
of inflammation, cancer, endocrine disorders and
20 gastrointestinal disorders.

The term, "substituted" as used herein and in the
claims, means that any one or more hydrogen atoms on the
designated atom is replaced with a selection from the
indicated group, provided that the designated atom's normal
25 valency is not exceeded, and that the substitution results in
a stable compound.

When any variable (for example R, X, Z, etc.) occurs
more than one time in any constituent or in any Formula
herein, its definition on each occurrence is independent of
30 its definition at every other occurrence. Also, combinations
of substituents and/or variables are permissible only if such
combinations result in stable compounds.

As used herein "peptide" indicates a sequence of amino
acids linked by peptide bonds. The somatostatin peptide
35 analogs of this invention comprise a sequence of amino acids
of 4 to 24 amino acid residues, preferably 6 to 14 residues,

each residue being characterized by having an amino and a
carboxy terminus.

A "building unit" indicates an N$^\alpha$ derivatized α amino
acid of the general Formula IV:

5

$$\text{————N-CH(R')-CO————}$$
$$|$$
$$|$$
$$|$$
$$X$$
$$|$$
10
$$|$$
$$|$$
$$\text{———G}$$
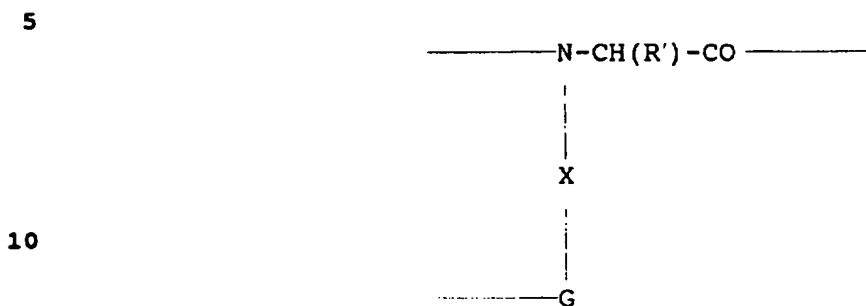
Formula (IV)

15 wherein X is a spacer group selected from the group
consisting of alkylene, substituted alkylene, arylene,
cycloalkylene and substituted cycloalkylene; R' is an amino
acid side chain, optionally bound with a specific protecting
group; and G is a functional group selected from the group
20 consisting of amines, thiols, alcohols, carboxylic acids and
esters, and alkyl halides; which is incorporated into the
peptide sequence and subsequently selectively cyclized via
the functional group G with one of the side chains of the
amino acids in said peptide sequence or with another ω-
25 functionalized amino acid derivative.

The methodology for producing the building units is
described in international patent application PCT/IB95/00455,
which is incorporated in its entirety by way of reference.
The building units are abbreviated by the three letter code
30 of the corresponding modified amino acid followed by the type
of reactive group (N for amine, C for carboxyl), and an
indication of the number of spacing methylene groups. For
example, Gly-C2 describes a modified Gly residue with a
carboxyl reactive group and a two carbon methylene spacer,
35 and Phe-N3 designates a modified phenylalanine group with an
amino reactive group and a three carbon methylene spacer.

As used herein "linear peptide" denotes the peptide sequence that is constructed only of amino acid residues and is devoid of any building units.

As used herein "backbone cyclic peptide" denotes an
5 analog of a linear peptide which contains at least one building unit that has been liked to form a bridge via the alpha nitrogen of the peptide backbone to another building unit, or to another amino acid in the sequence.

As used herein "pre-cyclic peptide" denotes an analog
10 identical to the cyclic analog except that it is retained in the non-cyclized form to serve as control during the biological or other screening assays. The term non-cyclic can be used interchangeably with the term pre-cyclic.

Certain abbreviations are used herein to describe this
15 invention and the manner of making and using it. For instance, AcOH refers to acetic acid, Ada refers to adamantanacetyl, Adac refers to adamantanecarbonyl, Alloc refer to allyloxycarbonyl, Boc refers to the t-butyloxycarbonyl radical, BOP refers to benzotriazol-1-yloxy-
20 tris-(dimethylamino)phosphonium hexafluorophosphate, BSA refers to bovine serum albumin, Cbz refers to the carbobenzyloxy radical, DCC refers to dicyclohexylcarbodiimide, DCM refers to Dichloromethane, Dde refers to 1-(4,4-dimethyl2,6-dioxocyclohex-1-ylidene-ethyl),
25 DIEA refers to diisopropyl-ethyl amine, DMF refers to dimethyl formamide, DPPA refers to diphenylphosphoryl azide, Dtc refers to 5,5-dimethylthiazolidine-4-carboxylic acid, EDC refers to N-ethyl-N' (dimethylaminopropyl)-carbodiimide, EDT refers to ethanedithiol, Fmoc refers to the
30 fluorenylmethoxycarbonyl radical, GPI refers to guinea pig ileum, HATU refers to [O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate, HBTU refers to 1-hydroxybenztriazolyltetramethyl-uronium hexafluorophosphate, HF refers to hydrofluoric acid, HOBT refers to 1-
35 hydroxybenzotriazole, HPLC refers to high pressure liquid chromatography, MALDI-TOF MS refers to matrix-assisted laser desorption, time-of-flight mass spectrometry, Mts refers to

the 4-methoxy-2,3,6-trimethylbenzenzsulfonyl, NBT refers to
nitro blue tetrazolium, NMM refers to N-methylmorpholine, NMP
refers to 1-methyl-2-pyrolidonone, PBS refers to phosphate
buffered saline, Pmc refers to pentamethylchroman-6-sulfonyl,
5 PNPP refers to p-nitrophenyl phosphate, PPA refers to 1-
propanephosphoric acid cyclic anhydride, PyBOP refers to
Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium
hexafluorophosphate, PyBrOP refers to Bromo-tris-
pyrrolidino-phosphonium hexafluorophosphate, RT refers to
10 room temperature, SMPS refers to simultaneous multiple
peptide synthesis, SRIF refers to Somatotropin Release
Inhibitory Factor, TBTU refers to 2-(1H-benzotriazole-1-yl)-
1,1,3,3-tetramethyluronium tetrafluoroborate, t-Bu refers to
the tertiary butyl radical, TFA refers to trifluoroacetic
15 acid, TIS refers to triisopropylsilane, Tpr refers to
thiazolidine-4-carboxylic acid, Trt refers to trityl, Ts
refers to toluenesulfonyl.

The amino acids used in this invention are those which
are available commercially or are available by routine
20 synthetic methods. Certain residues may require special
methods for incorporation into the peptide, and either
sequential, divergent and convergent synthetic approaches to
the peptide sequence are useful in this invention. Natural
coded amino acids and their derivatives are represented by
25 three-letter codes according to IUPAC conventions. When
there is no indication, the L isomer was used. The D isomers
are indicated by "D" before the residue abbreviation. List
of Non-coded amino acids: Abu refers to 2-aminobutyric acid,
Aib refers to 2-amino-isobutyric acid, Cha refers to
30 cyclohexylalanine, Hcys refer to homocystein, Hyp refers to
S-trans-4-hydroxyproline, 1Nal refers to 1-naphthylalanine,
2Nal refers to 2-naphtylalanine, Nva refers to norvaline,
Oic refers to octahydroindolecarboxylic acid, Phg refers to
phenylglycine, pClPhe refers to p-chloro-phenylalanine,
35 pFPhe refers to p-fluoro-phenylalanine, pNO₂Phe refers to
p-nitro-phenylalanine, Thi refers to thienylalanine.

Synthetic Approaches

According to the present invention peptide analogs are cyclized via bridging groups attached to the alpha nitrogens of amino acids that permit novel non-peptidic linkages. In

5 general, the procedures utilized to construct such peptide analogs from their building units rely on the known principles of peptide synthesis; most conveniently, the procedures can be performed according to the known principles of solid phase peptide synthesis. The innovation requires

10 replacement of one or more of the amino acids in a peptide sequence by novel building units of the general Formula:

$$HN-CH(R)-COOH$$
$$|$$
$$X$$
$$|$$
$$G$$

15
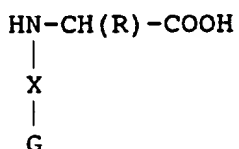
wherein R is the side chain of an amino acid, X is a spacer group and G is the functional end group by means of which cyclization will be effected. The side chain R is the side chain of any natural or synthetic amino acid that is selected

20 to be incorporated into the peptide sequence of choice. X is a spacer group that is selected to provide a greater or lesser degree of flexibility in order to achieve the appropriate conformational constraints of the peptide analog. Such spacer groups include alkylene chains, substituted,

25 branched and unsaturated alkylenes, arylenes, cycloalkylenes, and unsaturated and substituted cycloalkylenes. Furthermore, X and R can be combined to form a heterocyclic structure.

A preferred embodiment of the present invention utilizes alkylene chains containing from two to ten carbon atoms.

30   The terminal (ω) functional groups to be used for cyclization of the peptide analog include but are not limited to:

a.   Amines, for reaction with electrophiles such as activated carboxyl groups, aldehydes and ketones (with or

35 without subsequent reduction), and alkyl or substituted alkyl halides.

- 27 -

b.   Alcohols, for reaction with electrophiles such as
activated carboxyl groups.

c.   Thiols, for the formation of disulfide bonds and
reaction with electrophiles such as activated carboxyl
5 groups, and alkyl or substituted alkyl halides.

d.   1,2 and 1,3 Diols, for the formation of acetals and
ketals.

e.   Alkynes or Substituted Alkynes, for reaction with
nucleophiles such as amines, thiols or carbanions; free
10 radicals; electrophiles such as aldehydes and ketones, and
alkyl or substituted alkyl halides; or organometallic
complexes.

f.   Carboxylic Acids and Esters, for reaction with
nucleophiles (with or without prior activation), such as
15 amines, alcohols, and thiols.

g.   Alkyl or Substituted Alkyl Halides or Esters, for
reaction with nucleophiles such as amines, alcohols, thiols,
and carbanions (from active methylene groups such as
acetoacetates or malonates); and formation of free radicals
20 for subsequent reaction with alkenes or substituted alkenes,
and alkynes or substituted alkynes.

h.   Alkyl or Aryl Aldehydes and Ketones for reaction with
nucleophiles such as amines (with or without subsequent
reduction), carbanions (from active methylene groups such as
25 acetoacetates or malonates), diols (for the formation of
acetals and ketals).

i.   Alkenes or Substituted Alkenes, for reaction with
nucleophiles such as amines, thiols, carbanions, free
radicals, or organometallic complexes.

30 j.   Active Methylene Groups, such as malonate esters,
acetoacetate esters, and others for reaction with
electrophiles such as aldehydes and ketones, alkyl or
substituted alkyl halides.

        It will be appreciated that during synthesis of the
35 peptide these reactive end groups, as well as any reactive
side chains, must be protected by suitable protecting groups.

Suitable protecting groups for amines are alkyloxy, substituted alkyloxy, and aryloxy carbonyls including, but not limited to, tert butyloxycarbonyl (Boc), Fluorenylmethyloxycarbonyl (Fmoc), Allyloxycarbonyl (Alloc)
5 and Benzyloxycarbonyl (Z).

Carboxylic end groups for cyclizations may be protected as their alkyl or substituted alkyl esters or thio esters or aryl or substituted aryl esters or thio esters. Examples include but are not limited to tertiary butyl ester, allyl
10 ester, benzyl ester, 2-(trimethylsilyl)ethyl ester and 9-methyl fluorenyl.

Thiol groups for cyclizations may be protected as their alkyl or substituted alkyl thio ethers or disulfides or aryl or substituted aryl thio ethers or disulfides. Examples of
15 such groups include but are not limited to tertiary butyl, trityl(triphenylmethyl), benzyl, 2-(trimethylsilyl)ethyl, pixyl(9-phenylxanthen-9-yl), acetamidomethyl, carboxymethyl, 2-thio-4-nitropyridyl.

It will further be appreciated by the artisan that the
20 various reactive moieties will be protected by different protecting groups to allow their selective removal. Thus, a particular amino acid will be coupled to its neighbor in the peptide sequence when the N^α is protected by, for instance, protecting group A. If an amine is to be used as an end
25 group for cyclization in the reaction scheme the N^ω will be protected by protecting group B, or an ε amino group of any lysine in the sequence will be protected by protecting group C, and so on.

The coupling of the amino acids to one another is
30 performed as a series of reactions as is known in the art of peptide synthesis. Novel building units of the invention, namely the N^α-ω functionalized amino acid derivatives are incorporated into the peptide sequence to replace one or more of the amino acids. If only one such N^α-ω functionalized
35 amino acid derivative is selected, it will be cyclized to a side chain of another amino acid in the sequence. For

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instance: (a) an N^u-(ω-amino alkylene) amino acid can be
linked to the carboxyl group of an aspartic or glutamic acid
residue; (b) an N^α -(ω-carboxylic alkylene) amino acid can be
linked to the ϵ- amino group of a lysine residue; (c) an N^α-
(ω-thio alkylene) amino acid can be linked to the thiol group
of a cysteine residue; and so on. A more preferred
embodiment of the invention incorporates two such N^α-
ω-functionalized amino acid derivatives which may be linked
to one another to form N-backbone to N-backbone cyclic
peptide analogs. Three or more such building units can be
incorporated into a peptide sequence to create bi-cyclic
peptide analogs as will be elaborated below. Thus, peptide
analogs can be constructed with two or more cyclizations,
including N-backbone to N-backbone, as well as backbone to
side-chain or any other peptide cyclization.

As stated above, the procedures utilized to construct
somatostatin analogs of the present invention from novel
building units generally rely on the known principles of
peptide synthesis. However, it will be appreciated that
accommodation of the procedures to the bulkier building units
of the present invention may be required. Coupling of the
amino acids in solid phase peptide chemistry can be achieved
by means of a coupling agent such as but not limited to
dicyclohexycarbodiimide (DCC), bis(2-oxo-3oxazolidinyl)
phosphinic chloride (BOP-Cl), benzotriazolyl-N-
oxytrisdimethyl-aminophosphonium hexafluoro phosphate (BOP),
1-oxo-1-chlorophospholane (Cpt-Cl), hydroxybenzotriazole
(HOBT), or mixtures thereof.

It has now been found that coupling of the subsequent
amino acid to the bulky building units of the present
invention may require the use of additional coupling reagents
including, but not limited to: coupling reagents such as
PYBOP® (Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium
hexafluorophosphate), PyBrOP® (Bromo-tris-pyrrolidino-
phosphonium hexafluorophosphate), HBTU (2-(1H-Benzotriazole-
1-yl)-1,1,3,3- tetramethyluronium hexafluoro-phosphate), TBTU

(2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate).

Novel coupling chemistries may be used, such as pre-formed urethane-protected N-carboxy anhydrides (UNCA'S)

5 and pre-formed acyl halides most preferably acy chlorides. Such coupling may take place at room temperature and also at elevated temperatures, in solvents such as toluene, DCM (dichloromethane), DMF (dimethylformamide), DMA (dimethylacetamide), NMP (N-methyl pyrrolidinone) or mixtures

10 of the above.

One object of the present invention is a method for the preparation of backbone cyclized somatostatin analogs of general Formula (I):
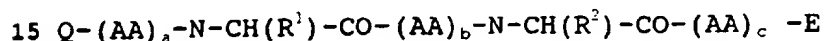
15 $Q-(AA)_a-N-CH(R^1)-CO-(AA)_b-N-CH(R^2)-CO-(AA)_c-E$

Formula (I)

20 wherein the substituents are as defined above;

comprising the steps of incorporating at least two $N^\circ-\omega$-functionalized derivatives of amino acids of Formula (III):
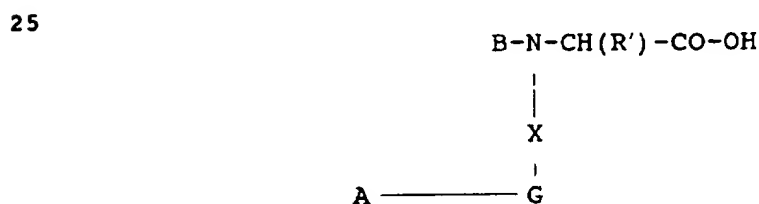
25

$$B-N-CH(R')-CO-OH$$
$$\begin{array}{c} | \\ X \\ | \\ A \underline{\hspace{2cm}} G \end{array}$$

30   Formula (III)

wherein X is a spacer group selected from the group consisting of alkylene, substituted alkylene, arylene, cycloalkylene and substituted cycloalkylene; R' is an amino

35 acid side chain such as H, CH_2 etc., optionally bound with a specific protecting group; B is a protecting group selected

from the group consisting of alkyloxy, substituted alkyloxy,
or aryloxy carbonyls; and G is a functional group selected
from the group consisting of amines, thiols, alcohols,
carboxylic acids and esters, aldehydes, alcohols and alkyl
5 halides; and A is a specific protecting group of G;

into an amino acid sequence to yield a compound of Formula:

$$B-(AA)_d-N-CH(R)-CO-(AA)_e-N-CH(R^U)-CO-(AA)_f-E$$

10
$$\begin{array}{cc} | & | \\ XU & X \\ | & | \\ AU\text{------}GU & A\text{------}G \end{array}$$

Formula (IV)

15

(ii) selectively removing protecting groups A and A'
and reacting the terminal groups G and G' to form a compound
of the Formula:

20
$$\overline{\quad\quad\quad\quad\quad\quad\quad}$$
$$| \quad\quad\quad\quad\quad\quad |$$
$$B-(AA)_d-N-CH(R)-CO-(AA)_e-N-CH(R^U)-CO-(AA)_f-E$$
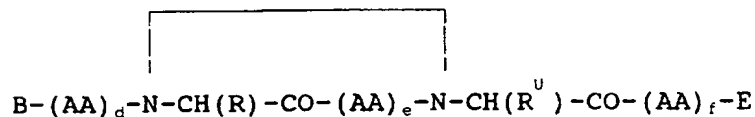
Formula (I)

25

wherein d, e and f are independently an integer from 1
to 10; (AA) is an amino acid residue wherein the amino acid
residues in each chain may be the same or different; E is an
hydroxyl group, a carboxyl protecting group or an amino
30 group; R and R' are independently an amino acid side-chain
such as H, CH_3, etc.; and the line designates a bridging group
of the Formula: -X-M-Y-W-Z-

wherein M and W are independently selected from the
group consisting of disulfide, amide, thioether, imine,
35 ether, and alkene; X, Y and Z are independently selected from

the group consisting of alkylene, substituted alkylene, arylene, cycloalkylene, and substituted cycloalkylene;

(iii) removing all remaining protecting groups to yield a compound of Formula (Ia).

Bicyclic analogs are prepared in the same manner, that is, by repetition of steps (ii) and (iii). The determination of which residues are cyclized with which other residues is made through the choice of blocking groups. The various blocking groups may be removed selectively, thereby exposing the selected reactive groups for cyclization.

Preferred are methods for the preparation of backbone cyclized peptide analogs of Formula (I) wherein G is an amine, thiol or carboxyl group; R and R^1 are each other than H, such as CH_3, $(CH_3)_2CH-$, $(CH_3)_2CHCH_2-$, $CH_3CH_2CH(CH_3)-$, $CH_3S(CH_2)_2-$, $HOCH_2-$, $CH_3CH(OH)-$, $HSCH_2-$, $NH_2C(=O)CH_2-$, $NH_2C(=O)(CH_2)_2-$, $HOC(=O)CH_2-$, $HOC(=O)(CH_2)_2-$, $NH_2(CH_2)_4-$, $C(NH_2)_2NH(CH_2)_3-$, $HO-phenyl-CH_2-$, benzyl, methylindole, and methylimidazole, and wherein E is covalently bound to an insoluble polymeric support.

Another object of the present invention is a method for the preparation of backbone cyclized peptide analogs of Formula (II):

$$H(AA)_d-N-CH(R^1)-CO-(AA)_e-NH-CH-CO-(AA)_f-E$$

Formula (II)

wherein the substituents are as defined above;

comprising the steps of: incorporating at least one ω-functionalized amino acid derivative of the general Formula (III):

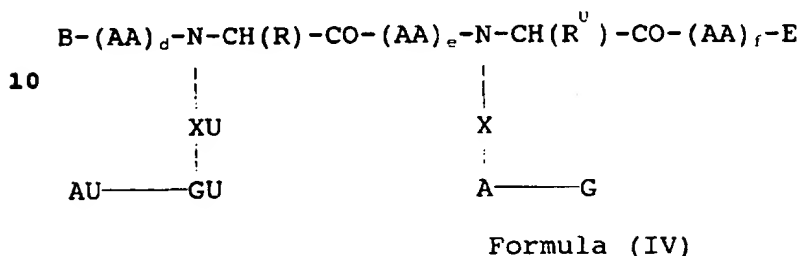$$B-N-CH(R')-CO-OH$$
$$\vert$$
$$X$$

$$A \text{——————} G$$

Formula (III)

wherein X is a spacer group selected from the group consisting of alkylene, substituted alkylene, arylene, cycloalkylene and substituted cycloalkylene; R is the side chain of an amino acid, such as H, CH_3, etc.; B is a protecting group selected from the group consisting of alkyloxy, substituted alkyloxy, or aryloxy carbonyls; and G is a functional group selected from the group consisting of amines, thiols, alcohols, carboxylic acids and e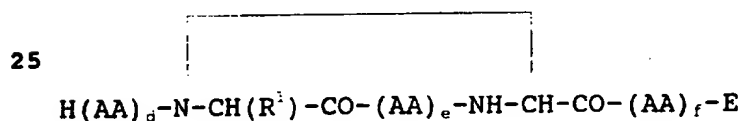sters or alkyl halides; and A is a protecting group thereof; into a peptide sequence and subsequently selectively cyclizing the functional group with one of the side chains of the amino acids in said peptide sequence.

Preferred is the method for the preparation of backbone cyclized peptide analogs of Formula (II) wherein G is a carboxyl group or a thiol group; R is CH_3, $(CH_3)_2CH-$, $(CH_3)_2CHCH_2-$, $CH_3CH_2CH(CH_3)-$, $CH_3S(CH_2)_2-$, $HOCH_2-$, $CH_3CH(OH)-$, $HSCH_2-$, $NH_2C(=O)CH_2-$, $NH_2C(=O)(CH_2)_2-$, $HOC(=O)CH_2-$, $HOC(=O)(CH_2)_2-$, $NH_2(CH_2)_4-$, $C(NH_2)_2NH(CH_2)_3-$, HO-phenyl-CH_2-, benzyl, methylindole, and methylimidazole, and wherein E is covalently bound to an insoluble polymeric support.

Preparation of Peptides with Backbone to Side Chain Cyclization.

One preferred procedure for preparing the desired backbone cyclic peptides involves the stepwise synthesis of the linear peptides on a solid support and the backbone cyclization of the peptide either on the solid support or after removal from the support. The C-terminal amino acid is bound covalently to an insoluble polymeric support by a carboxylic acid ester or other linkages such as amides. An example of such support is a polystyrene-co-divinyl benzene resin. The polymeric supports used are those compatible with

such chemistries as Fmoc and Boc and include for example PAM
resin, HMP resin and chloromethylated resin. The resin bound
amino acid is deprotected for example with TFA and to it is
coupled the second amino acid, protected on the N^α for example
5 by Fmoc, using a coupling reagent like BOP. The second amino
acid is deprotected using for example piperidine 20% in DMF.
The subsequent protected amino acids can then be coupled and
deprotected at ambient temperature. After several cycles of
coupling and deprotection that gives peptide, an amino acid
10 having for example carboxy side chain is coupled to the
desired peptide. One such amino acid is Fmoc-aspartic acid
t-butyl ester. After deprotection of the N^α Fmoc protecting
group, the peptide is again elongated by methods well known
in the art. After deprotection a building unit for backbone
15 cyclization is coupled to the peptide resin using for example
the coupling reagent BOP. One such building unit is for
example Fmoc-N^α-(ω-Boc-amino alkylene)amino acid. After
deprotection the peptide can then be elongated, to the
desired length using methods well known in the art. The
20 coupling of the protected amino acid subsequent to the
building unit is performed by such coupling agents
exemplified by PyBrOP to ensure high yield. After the
linear, resin bound peptide, has been prepared the co-
alkylene-protecting groups, for example Boc and t-Bu, are
25 removed by mild acid such as TFA. The resin bound peptide is
then divided into several parts. One part is subjected to
on-resin cyclization using for example TBTU as cyclization
agent in DMF to ensure high yield of cyclization, to give the
N-backbone to side chain cyclic peptide resin. After
30 cyclization on the resin the terminal amino protecting group
is removed by agents such as piperidine and the backbone to
side chain cyclic peptide is obtained after treatment with
strong acid such as HF. Alternatively, prior to the removal
of the backbone cyclic peptide from the resin, the terminal
35 amino group is blocked by acylation with agents such as
acetic anhydride, benzoic anhydride or any other acid such as

adamantyl carboxylic acid activated by coupling agents such as BOP.

The other part of the peptide-resin undergoes protecting of the side chains used for cyclization, for example the ω-

5 amino and carboxy groups. This is done by reacting the ω-amino group with for example Ac_2O and DMAP in DMF and activating the free ω-carboxy group by, for example, DIC and HOBT to give the active ester which is then reacted with, for example, CH_3NH_2 to give the non-cyclic analog of the cyclic

10 peptide. Removal of the peptide from the resin and subsequent removal of the side chains protecting groups by strong acid such as HF to gives the non-cyclic analog of the backbone to side chain cyclic peptide.

The linear and/or non-cyclic analogs are used as

15 reference compounds for the biological activity of their corresponding cyclic compounds.

Synthetic Approach for Generation of Backbone Cyclized Somatostatin Libraries.

20   The general methodology for preparing the cyclic peptide libraries of this invention involves solid phase peptide synthesis using an orthogonal protection scheme which allows for chain elongation, selective removal of the protecting groups, cyclization of the protected peptides and removal of

25 all side-chains protecting groups with or without cleavage from the resin. It is desirable that the various peptide sequences be present in the libraries in substantially equal amount.

The coupling reactions are performed by methods to

30 create amide or ester bonds and are performed by methods familiar in the art as described herein. Typical coupling reagents are carbodiimides, activated anhydrides and esters and acyl halides. Reagents such as EDC, DCC, DPPA, PPA, BOP, PyBOP, PyBrop, HATU, HBTU, TBTU, HOBT and

35 Ṅ-hydroxysuccinimide are typical.

After completion of the solid phase peptide elongation,
by any scheme, portions of the peptide are cyclized via the
bridging groups attached to the backbone amine bond nitrogens
of the building units. It is preferable that a portion is
5 retained in the non-cyclized form to serve as control during
the biological or other screening assays. This portion of
the peptide analog library, which contains the building units
identical to those of the backbone cyclized library, but is
devoid of the conformational constraint of the latter, is
10 referred to as the "pre-cyclic". Alternatively, in any of
the synthesis schemes, the backbone cyclization step may be
performed and additional coupling cycles of amino acid
residues may then be carried out.

    Portions of the peptide may be cleaved from the resin
15 and protecting groups removed, as required prior to assay of
biological activity. The peptides are cleaved from the resin
support by methods known in the art, the precise method being
dependent upon the characteristics of the resin. It will be
understood by those skilled in the art that the removal of
20 certain protecting groups may occur simultaneously with
cleavage of the peptide from the resin.

    Typically the coupling between the resin and the first
amino acid will form an ester bond, which will yield a
carboxylic acid group on the peptide when it is cleaved from
25 the resin. HMPB, Rink, PAM, Hycram and hydroxymethyl resins
are exemplary. In addition, the carboxy terminal amino acid
group may be converted to an amide, an ester or reduced to a
terminal alcohol.

    The reactive functional groups of the side chains of
30 each amino acid or peptide are suitably protected as known in
the peptide art. For example, the Boc, Cbz or Fmoc group may
be used for protection of an amino group, especially an α-
amino group. An alkyl (e.g., t-Bu, Me), cHex, benzyl or
allyl ester may be used for the protection of the side chain
35 carboxyl of Asp or Glu. A benzyl, or suitably substituted
benzyl, trityl, Alloc or T-Bu group is used to protect the
mercapto group of cysteine, or other thiol containing

residues; or the hydroxyl of Tyr, Ser or Thr. Cys and other
sulfur-containing amino acids may also be protected by the
Acm group or by formation of a disulfide with a thioalkyl
(e.g., ethyl mercaptan) or thioaryl group. The
5 benzyl/benzyloxymethyl, or a suitably substituted
benzyl/benzyloxymethyl, Boc or formyl group may be used for
protection of the imidazolyl group of His; and the Pmc,
nitro, or a suitably substituted benzene-sulfonyl group
(e.g., Ts, Mts) for protection of the guanidino nitrogen or
10 Arg. The phthalamido, Boc, Fmoc, Alloc carbobenzyloxy or
benzyl group, or suitably substituted benzyl or benzyloxy
group, may be used for protecting the e-amino group of
lysine. Suitable substitution of the carbobenzyloxy or
benzyl protecting groups is substitution with one to five
15 chloro, bromo, nitro, methoxy or methyl groups, usually ortho
and/or para, and is used to modify the reactivity of the
protective group. These protective groups are removed by
such methods as catalytic hydrogenation, sodium in liquid
ammonia, hydrazine, base, TFA or HF treatment, as known in
20 the art. The choice of side chain protecting groups is
chosen so that they will not be removed under conditions
which are used to deprotect the reactive functional group
used in the coupling reaction (e.g., generally the α-amino
group) to form the peptide backbone of the peptide chain.
25 The protective group of the reactive functional group is
removed prior to coupling each successive amino acid.

The bridging groups of the building units (i.e., G in
Formula IV) are used according to the present invention with
an orthogonal protection scheme, such that these protecting
30 groups can be removed selectively, under conditions which do
not affect the protecting groups on the side chains or
cleavage of the peptide from the resin. This enables
backbone cyclization on the resin, which is preferred
synthetically. Alternatively, the fully protected peptide
35 may be removed from the resin, and cyclization performed in

solution after selective removal of the protecting groups of
the building units.

The cyclization reaction is carried out by means of
selectively coupling the bridging group of one building unit
5 to a bridging group of another building unit or amino acid
side chain. By way of example, PyBOP is a particularly
useful reagent for conducting the coupling reaction, in case
of formation of an amide bond. To form a disulfide bridge
oxidative conditions are used.

10 In a most preferred embodiment according to the present
invention, the amino acid sequence scaffold is based on known
active sequences from natural or synthetic peptides having
somatostatin activity. It will thus be possible to further
improve the activity of such known sequences upon
15 rigidification of the active conformer.

Amino acids in certain positions are replaced by
Backbone-Cyclization Building-Units or by natural and non-
natural trifunctional amino acids such as Asp, Glu, Cys,
Hcys, Lys, Orn and their D counterparts. Thus positional as
20 well as structural scans are performed by changing the
position of cyclization, the link of the ring to the
backbone, the chirality at the position of cyclization, the
ring forming bond, the ring size and the exact placement of
the bond within the ring. These variations may also be
25 performed in conjunction with changing the amino acid
sequence of the peptide.

General synthesis of libraries of somatostatin analogs.

To determine the optimum compounds, a library of
30 differently constrained analogs is generated and then
screened. The libraries were synthesized on TentaGel amide
Resin (substitution level of 0.2-0.3 mmol/g) using
conventional solid-phase peptide synthesis (known to those
skilled in the art). In most cases NMP was used as a
35 solvent, DMF in few cases. Synthesis scale was 0.2-2 μmole
for each peptide in library or sub-library. Unless otherwise
mentioned, all reactions were performed at room temperature.

In each coupling step where more than one amino acid had
to be coupled, the resin was divided into the appropriate
number of portions and different amino acid was added to each
portion. Coupling was performed, twice for each position
5 with 3 molar excess of each amino acid, 3 molar excess of
PyBrop and 6 molar excess of DIEA for duration of 1-16 hours.
All amino acids were protected with FMOC in their α-amine.
Side-chain protections were as follow: His(Trt); Lys(Boc or
Dde); Orn(Boc); Ser(tBu); Thr(tBu); Tyr(tBu).

10   After double coupling, the resin portions were washed,
recombined and FMOC deprotection was performed using 20%
piperidine in NMP for total of 20-40 minutes. After
additional washes the resin was divided again (if necessary)
for the coupling of the next amino acid/s.

15   Before cyclization, the Allyl/Alloc protection of the
amine and carboxyl of the building units were removed by
treatment with a solution of 2 mole equivalents (one for each
Allyl/Alloc molecule in peptide), of Pd(PPh3)$_4$ dissolved in
chloroform containing 2.5% AcOH and 5% NMM for 2-2.5 hours or
20 twice for 1 hour, resins were washed with the above solvent
without the palladium before and after treatment, additional
washes with NMP were made at the end of the removal process.

       The peptides were cleaved from the resin portions after
washes with DCM, by double treatment with TFA 70%, H_2O 5%, TIS
25 1%, EDT 2.5%, DCM (mixture A) or TFA 70%, H_2O 5%, TIS 1%,
Phenol 5%, DCM (mixture B) or 60% TFA, 10% H_2O and 30% DCM
(mixture C) plus additional wash with neat TFA. The three
cleavage solutions of each resin portion were collected
together, evaporated with nitrogen stream, 0.5-1 ml of H_2O
30 were added to each sample that was then freeze-dried. The
peptide mixtures were then partially purified on C-18 SEP-PAK
(Millipore Corp.) using 0.1% acetic acid or TFA in H_2O as
buffer A and 50-80% CH_3CN in 0.1% acetic acid / H_2O as buffer
B and freeze-dried.

35   Each sub-library synthesized is characterized by mass
spectrometry (MALDI-TOF MS), and amino acid analysis.

The building units are abbreviated by the three letter
code of the corresponding modified amino acid followed by the
type of reactive group (N for amine, C for carboxyl), and an
indication of the number of spacing methylene groups. For
5 example, Gly-C2 describes a modified Gly residue with a
carboxy reactive group and a two carbon methylene spacer, and
Phe-N3 designates a modified phenylalanine group with a amino
reactive group and three carbon methylene spacer.

10 General screening of somatostatin analogs
    Somatostatin analogs synthesized are typically tested in
vitro for their inhibition of the natural peptide (SRIF-14)
binding to its 7-transmembranal receptors, and for their
influence on second messengers and cell growth; and in vivo
15 for inhibition of hormones and enzyme secretion.
    The analogs are further tested in vitro for their
influence on cyclic adenosine monophosphate (cAMP) levels,
tyrosine phosphatase activity, growth hormone secretion, and
cell growth. The libraries are further tested in vivo for
20 the inhibition of growth-hormone release, and amylase,
gastric acid, insulin and glucagon secretion in animals.

Metabolic stability tests as parameter for selection:
    Analogs are tested for stability by their resistance to
25 enzymatic degradation by incubation in serum or in tissue
homogenate, separation of the proteins and recording of the
peptide peaks by HPLC before and after incubation. The
peptide peaks that are not changed with increased incubation
time are most stable. These peaks are separated and
30 characterized by mass spectrometry, N-terminal sequence and
comparison to purified peptide peaks. In this way the most
stable peptides from library or sub-library are rapidly
identified.
    Conformationally constrained somatostatin analogs
35 constructed based in part on the sequences of a number of
known biologically active peptides or based on previously
unknown novel sequences are presented in the examples below.

The following examples are intended to illustrate how to make and use the compounds and methods of this invention and are in no way to be construed as a limitation.

5   **EXAMPLES**

Synthetic examples

Different series of somatostatin analogs were synthesized, either as individual backbone cyclized peptides 10 or as libraries.

Three series of octapeptide somatostatin analogs corresponding to general Formula (Va) of the present invention were individually synthesized, characterized, and tested for biological activity.

15   1)   The first series of compounds corresponds to the general Formula (Va), wherein R^5 is (D)Phe; R^7 is Phe; R^{10} is Thr; and R^{12} is Thr. This series, therefore, comprises compounds of the specific formula:

$$H-(D)Phe-R^6-Phe-(D)Trp-Lys-Thr-R^{11}-Thr-NH_2$$
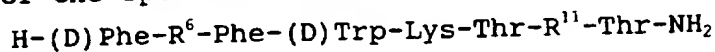
20 wherein R^6 and R^{11} are N^α ω-functionalized alkylene amino acid building units.

2)   The second series of compounds corresponds to the general Formula (Va), wherein R^5 is (D)Phe; R^7 is Phe; R^{10} is absent; and R^{12} is Thr. This series, therefore, comprises 25 compounds of the specific formula

$$H-(D)Phe-R^6-Phe-(D)Trp-Lys-R^{11}-Thr-NH_2$$
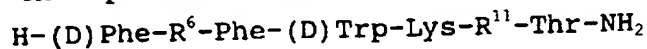
wherein R^6 and R^{11} are N^α ω-functionalized alkylene amino acid building units.

3)   The third series of compounds corresponds to the 30 general Formula (Va), wherein R^5 is (D)Phe; and R^7 is Phe. This series, therefore, comprises compounds of the specific formula

$$H-(D)Phe-R^6-Phe-(D)Trp-Lys-R^{10}-R^{11}-R^{12}-NH_2$$
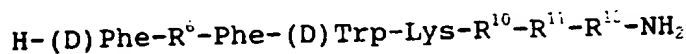
wherein R^6 and R^{11} are N^α ω-functionalized alkylene amino acid 35 building units.

The structures of these novel synthetic peptide analogs
into which N^α ω-functionalized amino acid building units were
incorporated, are summarized in Tables 1, 2 and 3. In these
three series, the building units used were glycine building
5 units in which the bridging groups, attached via the alpha
nitrogens to the peptide backbone, were varied.

For the sake of simplicity, these three series are
referred to herein as the SST Gly^6, Gly^{11}; SST Gly^6, Gly^{10}; and
SST Gly^6 Gly^{12} R^{10} R^{12}, respectively.

10   In each series, the position of the cyclization points
was constant; in the first and second series the length and
direction of the bridge was varied, whereas in the third
series the bridge was constant and residues at positions 10
and 12 were varied. Thus, C2, N2 refers to a bridge
15 consisting of an amide bond in which the carbonyl group is
closer to the amino end of the peptide and which contains a
two carbon methylene group between the bridge amide and each
of the backbone nitrogens involved in the bridge.

Peptide assembly was carried out either manually or with
20 an automatic peptide synthesizer (Applied Biosystems Model
433A). Following peptide assembly, de-protection of bridging
groups that form the cyclization arms was carried out with
$Pd(PPh_3)_4$ (palladium tetrakis triphenyl phosphine) in the case
of Allyl/Alloc protecting groups or with TFA in the case of
25 tBu/Boc protecting groups. For obtaining the non-cyclic
analog, the peptides were cleaved from the resin at this
stage. Cyclization of the peptides was carried out with
PyBOP. Cleavage of the peptides from the polymeric support
was carried out with suitable reagents depending on the type
30 of resin used, e.g., with TFA for Rink amide type resins and
with HF for mBHA (para-methyl benzhydryl amine) type resins.
The crude products were characterized by analytical HPLC.
The peptides were purified by preparative reversed phase
HPLC. The purified products were characterized by analytical
35 HPLC, mass spectroscopy, and amino acid analysis.

Table 1 SST Gly^6,Gly^{11} analogs

- 43 -

Example No.	Bridging Groups	Compound Number	Method	Crude Yield
1	C1,N2 Cyclic	DE-3-32-4	1	NA**
2	C1,N2 Non-cyclic	DE-3-32-2	1	NA
3	C1,N3 Cyclic	PTR 3004	2	79 mg
4	C1,N3 Non-cyclic	PTR 3005	2	34 mg
5	C2,N2 Cyclic	PTR 3002	1	NA
6	C2,N2 Non-cyclic	PTR 3001	1	NA
7	C2,N3 Cyclic	PTR 3007	2	40 mg
8	C2,N3 Non-cyclic	PTR 3008	2	40 mg
9	N2,C2 Cyclic	YD-9-166-1	2	NA
10	N2,C2 Non-cyclic	YD-9-168-1	2	NA
11	N3,C2 Cyclic	PTR 3010	2	100 mg
12	N3,C2 Non-cyclic	PTR 3011	2	NA
13	Linear*	PTR 3003	3	96 mg

* Linear refers to the identical sequence with underivatized Gly residues in place of R^6 and R^{11}.
** NA denotes not available.

Table 1 methods:
1) Manual synthesis on mBHA resin. HF cleavage.
2) Manual synthesis on Rapp TentaGel resin. TFA cleavage.
3) Rink amide resin; assembly in automated peptide synthesizer, 0.1 mmol scale.

Table 2 SST Gly^6,Gly^{10} analogs

Example No.	Bridging Groups	Compound Number	Method	Crude Yield
14	C1,N2 Cyclic	YD-9-171-3	1	20 mg
15	C1,N2 Non-cyclic	YD-9-171-2	1	10 mg
16	C1,N3 Cyclic	YD-9-175-3	1	44.9 mg
17	C1,N3 Non-cyclic	YD-9-175-2	1	25.4 mg
18	C2,N2 Cyclic	PTR 3019	1	40 mg
19	C2,N2 Non-cyclic	PTR 3020	1	26 mg
20	C2,N3 Cyclic	YD-5-28-3	3	101.5 mg
21	C2,N3 Non-cyclic	YD-5-28-2	3	48.3 mg
22	N2,C2 Cyclic	PTR 3016	2	60 mg
23	N2,C2 Non-cyclic	PTR 3017	2	40 mg
24	N3,C2 Cyclic	YS-8-153-1	2	93 mg
25	N3,C2 Non-cyclic	YS-8-152-1	2	54 mg

26	* Linear	PTR 3021	1	100 mg
27	N3,C2 Cyclic**	PTR 3013		67 mg
28	N3,C2 Non-cyclic**	PTR 3014		48 mg

* Linear refers to the identical sequence with Gly
  residues in place of R^6 and R^{10}.
** These analogs comprise of the same SST sequence in which
  the N terminal D-Phe^5 is absent and the N-terminus is
  acetylated.

Table 2 methods:
           1) Assembly in automated peptide synthesizer, 0.1
mmol scale.   (HBTU).
           2) Manual synthesis; PyBrop.
           3) Assembly in automated peptide synthesizer, 0.25
mmol scale.   (HBTU).

Table 3. Somatostatin analogs based on: H-(D)Phe-R^6-Phe-(D)Trp-Lys-R^{10}-R^{11}-R^{12}-NH_2

Example No.	Analog	R^{10}	R^{12}	Yield (mg)
29	GGP-22-65	Nva	Thr	390
30	GGP-22-63	Val	Thr	300
31	GGP-22-61	Abu	Thr	340
32	GGP-22-59	Ser	Thr	350
33	GGP-22-75	Thr	Nal	125
34	GGP-22-81	Val	Nal	210
35	GGP-22-82	Abu	Nal	200
36	GGP-22-77	Ser	Nal	190
37	GGP-22-83	Thr	(D)Nal	68
38	GGP-22-89	Val	(D)Nal	58
39	GGP-22-87	Abu	(D)Nal	65
40	GGP-22-85	Ser	(D)Nal	58

Example 41.   Detailed synthesis of SST Gly^6, Gly^{10} N3,C2 analog.

Five grams of Rink amide resin (NOVA) (0.49 mmol/g), were swelled in N-methylpyrrolidone (NMP) in a reaction vessel equipped with a sintered glass bottom and placed on a shaker. The Fmoc protecting group was removed from the resin by reaction with 20% piperidine in NMP (2 times 10 minutes, 25 ml each). Fmoc removal was monitored by ultraviolet absorption measurement at 290 nm. A coupling cycle was carried out with Fmoc-Thr(OtBu)-OH (3 equivalents) PyBrop (3 equivalents) DIEA (6 equivalents) in NMP (20 ml) for 2 hours at room temperature. Reaction completion was monitored by the qualitative ninhydrin test (Kaiser test). Following coupling, the peptide-resin was washed with NMP (7 times with 25 ml NMP, 2 minutes each). Capping was carried out by reaction of the peptide-resin with acetic anhydride (capping mixture: HOBt 400 mg, NMP 20 ml, acetic anhydride 10 ml, DIEA 4.4 ml) for 0.5 hours at room temperature. After capping, NMP washes were carried out as above (7 times, 2 minutes each). Fmoc removal was carried out as above. Fmoc-

Phe-OH was coupled in the same manner, and the Fmoc group
removed, as above. The peptide resin was reacted with Fmoc-
Gly-C2 (Allyl) building unit: coupling conditions were as
above. Fmoc removal was carried out as above. Fmoc-
5   Lys(Boc)-OH was coupled to the peptide resin by reaction with
HATU (3 equivalents) and DIEA (6 equivalents) at room
temperature overnight and then at 50E for one hour.
Additional DIEA was added during reaction to maintain a basic
medium (as determined by pH paper to be about 9). This
10 coupling was repeated. Coupling completion was monitored by
the Fmoc test (a sample of the peptide resin was taken and
weighed, the Fmoc was removed as above, and the ultraviolet
absorption was measured). Fmoc-D-Trp-OH was couped to the
peptide resin with PyBrop, as described above. Following
15 Fmoc removal, Fmoc-Phe-OH was coupled in the same way.
Synthesis was continued with one-fifth of the peptide resin.
        Following Fmoc removal, the second building unit was
introduced: Fmoc-Gly-N3(Alloc)-OH by reaction with PyBrOP, as
described above. Capping was carried out as described above.
20 Following Fmoc removal, the peptide-resin was divided into
two equal portions. Synthesis was continued with one of
these portions. Boc-D-Phe-OH was coupled by reaction with
HATU, as described above for Fmoc-Lys(Boc)-OH. Capping was
carried out as above.
25   The Allyl and Alloc protecting groups were removed by
reaction with $Pd(PPh_3)_4$ and acetic acid 5%, morpholine 2.5% in
chloroform, under argon, for 2 hours at room temperature.
The peptide resin was washed with NMP as above. Two-thirds
of the resin were taken for cyclization. Cyclization was
30 carried out with PyBOP 3 equivalents, DIEA 6 equivalents, in
NMP, at room temperature overnight. The peptide resin was
washed and dried. The peptide was cleaved from the resin by
reaction with TFA 81.5%, phenol 5%, water 5%, EDT 2.5%, TIS
(tri-isopropyl-silane) 1%, and 5% methylene chloride, at OEC
35 for 15 minutes and 2 hours at room temperature under argon.
The mixture was filtered into cold ether (30 ml, OEC) and the

resin was washed with a small volume of TFA. The filtrate
was placed in a rotary evaporator and all the volatile
components were removed. An oily product was obtained. It
was triturated with ether and the ether decanted, three
5 times. A white powder was obtained. This crude product was
dried. The weight of the crude product was 93 mg.

Additional series of novel backbone cyclized somatostatin
analogs according to Formula Vb were synthesized individually
10 including the following:

1)   heptapeptide series:
          NPhe-Tyr-(D)Trp-Lys-Val-NPhe-Thr-NH$_2$

15 2)   heptapeptide series:
          NPhe-Phe-(D)Trp-Lys-R^{10}-NPhe-R^{12}-NH$_2$
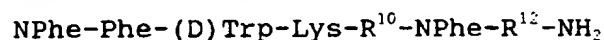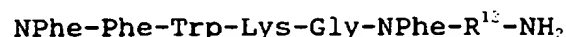
3)   Heptapeptides series:
          NPhe-Phe-Trp-Lys-Gly-NPhe-R^{12}-NH$_2$
20

In the first series (table 4), the length and direction of
the bridge was varied, whereas in the second (table 5), and
third (table 6), series the residues at positions 10 and/or
12 were varied.
25

30

35

Table 4. Heptapeptide somatostatin analogs based on:
R^6-Tyr-(D)Trp-Lys-Val-R^{11}-Thr-NH_2

Example No.	Analog	R^6	R^{11}	Yield (mg)
42*	GGP-22-151	Phe-N2	Phe-C3	290
43	GGP-22-135	Phe-N3	Phe-C3	25
44	GGP-22-159a	Phe-N2	Phe-C2	28
45	GGP-22-159b	Phe-N3	Phe-C2	30
46	GGP-22-161a	Phe-C2	Phe-N2	56
47	GGP-22-161b	Phe-C3	Phe-N2	65
48	GGP-22-163a	Phe-C1	Phe-N3	61
49	GGP-22-163b	Phe-C1	Phe-N3	68
50	GGP-22-163c	Phe-C3	Phe-N3	10

*PTR-3046

Table 5. Additional heptapeptide somatostatin analogs based
on: R^6-Phe-(D)Trp-Lys-R^{10}-R^{11}-R^{12}-NH_2

Example No.	Analog	R^6	R^{10}	R^{11}	R^{12}	Yield (mg)
51	GGP-22-53	Phe-N2	Val	Phe-C3	Val	200
52	GGP-22-55	Phe-N2	Val	Phe-C3	Thr	460
53	GGP-22-41	Phe-N2	Thr	Phe-C3	Thr	120
54	GGP-22-137	Phe-N3	Thr	Phe-C3	Thr	30
55	GGP-22-37	Phe-N2	Thr	Phe-C3	Val	146

Table 6. Heptapeptide somatostatin analogs based on: R^6-Phe-
Trp-Lys-Gly-R^{11}-R^{12}-NH_2

Example No.	R^6	7	R^8	R^9	R^{10}	R^{11}	R^{12}
56	Phe-N2	Phe	Trp	Lys	Gly	Phe-C3	Thr
57	Phe-N2	Phe	Trp	Lys	Gly	Phe-C3	Val
58	Phe-N2	Phe	Trp	Lys	Gly	Phe-C3	Ala
59	Phe-N2	Phe	Trp	Lys	Gly	Phe-C3	b-Ala
60	Phe-N2	Phe	Trp	Lys	Gly	Phe-C3	D2Nal

Example 61. Detailed synthesis of PTR 3046

    1g Rink Amide MBHA resin (NOVA) (0.55 mmol/g), were
swelled for 1.5h in NMP in a reaction vessel equipped with a
sintered glass bottom and placed on a shaker. The Fmoc
5 protecting group was removed from the resin by reaction with
20% Piperidine in NMP (2 times 15 minutes, 5 ml each). Fmoc
removal was monitored by Ninhydrin test. A coupling cycle
was carried out with Fmoc-Thr(OtBu)-OH (4 equivalents) PyBrop
(4 equivalents) DIEA (12 equivalents) in NMP (5 ml) for 0.5
10 hour at room temperature. Reaction completion was monitored
by the qualitative Ninhydrin test (Kaiser test). Following
coupling the peptide-resin was washed with NMP (3 times with
5 ml NMP, 5 ml DCM and 5 ml NMP for 2 minutes). Capping was
carried out by reaction of the peptide-resin with acetic
15 anhydride (capping mixture: HOAt 40 mg, NMP 5 ml, acetic
anhydride 1 ml, DIEA 0.5 ml and DMAP(cat)) for 0.5 hours at
room temperature. After capping NMP washes were carried out
as above). Fmoc removal was carried out as above. Fmoc-
Phe(C3)-Allyl BU was coupled(BU 2eq., PyBrop 2 eq. DIEA 6
20 eq., NMP 5 ml, 0.5 h. Fmoc removal was carried out as above.
The peptide resin was washed as above. The peptide resin was
reacted with Fmoc-Val-Cl (4 eq., Colidine 12 eq., 1h, 38C) by
double coupling. Coupling completion was monitored due to
conversion of dipeptide to tripeptide (a sample of the
25 peptide resin was cleaved and crude tripeptide was injected
into HPLC (0.1% water/acetonitrile). Fmoc removal was
carried out as above. Fmoc-Lys(Boc)-OH was coupled to the
peptide resin by reaction conditions as for Fmoc-Thr(OtBu)-OH
(see above). Coupling completion was monitored by Ninhydrin
30 test. Fmoc-D-Trp-OH was coupled to the peptide resin with
PyBrop, as described above. Following Fmoc removal, Fmoc-
Tyr(tBu)-OH was coupled in the same way. Following Fmoc
removal, the second building unit was introduced: Fmoc-Phe-
N2(Alloc)-OH by reaction with PyBrop, as described for Fmoc-
35 Phe(C3)-Allyl B. The Allyl and Alloc protecting groups were
removed by reaction with Pd(PPh₃)₄ and acetic acid 5%, N-
methylmorpholine 2.5% in chloroform, under argon, for 1.5

hours at room temperature. The peptide resin was washed as
above. Cyclization was carried out with PyBOP, 3
equivalents, DIEA 6 equivalents, in NMP, at room temperature
for 0.5h. The peptide resin was washed as above. Following
5 Fmoc removal the peptide resin was washed (DCM 3 x 5 ml),
dried and cleaved from the resin by reaction with TFA 94%,
water 2.5%, EDT 2.5%, TIS (tri-isopropyl-silane) 1%, at 0°C
for 15 minutes and 1.5 hours at room temperature. The
mixture was filtered and the resin was washed with a small
10 volume of TFA. The filtrate was placed in a rotary
evaporator and all the volatile components were removed. An
oily product was obtained. It was triturated with ether and
the ether decanted. Yellow powder was obtained. This crude
product was dried. The weight of the crude product was 290
15 mg.

Additional examples of individual somatostatin analogs

Additional individual examples of novel somatostatin analogs
20 produced according to the invention are summarized in
Table 7.

Table 7. Additional somatostatin analogs synthesized.

Example No.	R^5	R^6	7	R^8	R^9	R^{10}	R^{11}	R^{12}
61		Phe-N2	Tyr	(D)Trp	Lys	Val	Phe-C3	
62		Phe-N2	Tyr	(D)Trp	Lys	Val	Phe-C3	
63		Phe-N2	pNO₂Phe	(D)Trp	Lys	Val	Phe-C3	Thr
64		Phe-N2	Tyr	(D)Trp	Lys	(D)Val	Phe-C3	Thr
65		Phe-N2	pClPhe	(D)Trp	Lys	Val	Phe-C3	Thr
66		Phe-N2	Nal	(D)Trp	Lys	Val	Phe-C3	Thr
67	(D)Phe	Phe-N2	Tyr	(D)Trp	Lys	(D)Val	Phe-C3	

35

Libraries of Somatostatin analogs

Position numbers of amino acids in the somatostatin
sequence are based on the native somatostatin peptide (SRIF-
14, Raynor et. al. ibid).

5

Example 68: VH-SST1 library

The library was designed to contain 40 backbone cyclic
peptides in five final sub-libraries, each containing 8
different peptides.

10   Before the last coupling step (R^5), the resin was split
into 5 portions, coupling was performed for each portion with
different AA and left as separate sub-libraries for the
following steps. These five portions are the sub-libraries
as described in the table 8 which indicates all the residues
15 used at each position for generation of the 40 analogs.

Table 8. The composition of VH-SST1 library

Sub-library	Residues per position							
	R^5	R^6	R^7	R^8	R^9	R^{10}	R^{11}	R^{12}
A B C D E	DPhe Pro Val Leu Gly	Gly-C2	Phe	DTrp Leu	Lys Pro	Gly Val	Gly-N2	Val

25 Example 69: IG-SST1 library

In this library, the bridge was constant between
position 6 with Gly-C2 and position 10 with Gly-N2. The
library contains 36 peptides in 4 sub-libraries, which differ
in their R^5 residue. The composition of this library is shown
30 in table 9.

35

Table 9. The composition of IG-SST1 library

Sub-library	Residues per position							
	R^5	R^6	R^7	R^8	R^9	R^{10}	R^{11}	R^{12}
A B C D	DPhe Phe Dtrp Trp	Gly-C2	Phe	DTrp Phe Leu	Lys	Gly-N2	Phe Ala Leu	Val

Example 70: YS-SST1 library

This library represents 16 analogs in 4 sub-libraries as described in table 10. The sub-libraries are defined by four different bridge groups (between position R^6 and position R^{11} or R^{10}), and each sub-library contains four analogs.

Table 10.   The composition of YS-SST1 library

Sub-library	Residues per position							
	R^5	R^6	R^7	R^8	R^9	R^{10}	R^{11}	R^{12}
A1-2	DPhe	Gly-C1 Gly-C2	Phe	DTrp	Lys	Thr	Gly-N2 Gly-N3	Thr
A3-4	DPhe	Gly-N2 Gly-N3	Phe	DTrp	Lys	Thr	Gly-C1 Gly-C2	Thr
B1-2	DPhe	Gly-C2 Gly-C2	Phe	DTrp	Lys	Gly-N2 Gly-N3	Phe	Thr
B3-4	DPhe	Gly-N1 Gly-N3	Phe	DTrp	Lys	Gly-C1 Gly-C2	Phe	Thr

Example 71: YS-SST2 library

The library contains 48 peptides in 4 sub-libraries. The sub-libraries differ in their R^7 residue and contain 12 peptides each. The composition of this library is shown in table 11.

Table 11.   The composition of YS-SST2 library.

Sub-library	Residues per position							
	R^5	R^6	R^7	R^8	R^9	R^{10}	R^{11}	R^{12}
A B C D	DPhe	Gly-N3	Phe Tyr pClPhe pNO$_2$Phe	DTrp	Lys	Thr Ser Val Abu	Gly-C2	Thr 2Nal D2Nal

Example 72: YS-SST3 library

The library contains 12 peptides in 2 sub-libraries. The sub-libraries differ in their R^6 building unit and contain 6 peptides each. The composition of this library is shown in table 12.

Table 12: The composition of YS-SST3 library

Sub-library	Residues per position							
	R^5	R^6	R^7	R^8	R^9	R^{10}	R^{11}	R^{12}
A B	DPhe Gly	Phe-N2 Phe-N3	Phe	DTrp	Lys	Thr Ser Gly	Phe-C2	Thr

Example 73: YS-SST4 library

This library contains 48 peptides in two sub-libraries. Sub-library B differs from A by the existence of Thr at R^5 position as described in Table 13.

Table 13. The composition of YS-SST4 library

Sub-library	Residues per position							
	R^{12}	R^{11}	R^{10}	R^9	R^8	R^7	R^6	R^5
A		Phe-C2	DVal DAla DLeu	DLys DOrn	Trp Thi	DTyr	Gly-N2 Gly-N3	Phe
B	Thr	Phe-C2	DVal DAla DLeu	DLys DOrn	Trp Thi	DTyr	Gly-N2 Gly-N3	Phe

Example 74: YS-SST5 A and B libraries

These libraries were designed for optimization of bridge size and direction between positions 6 and 11 with simultaneous determination of the influence of various Naphtylalanine residues at position 5. The YS-SST-5A library consists of 4 sub-libraries with 16 peptides in each. The YS-SST-5B library, represents a simplified synthetic scheme of sub-libraries C and D, consisting of 8 sub-libraries of 4 peptides each. The library synthesis is illustrated in the following scheme.

YS5A LIBRARY
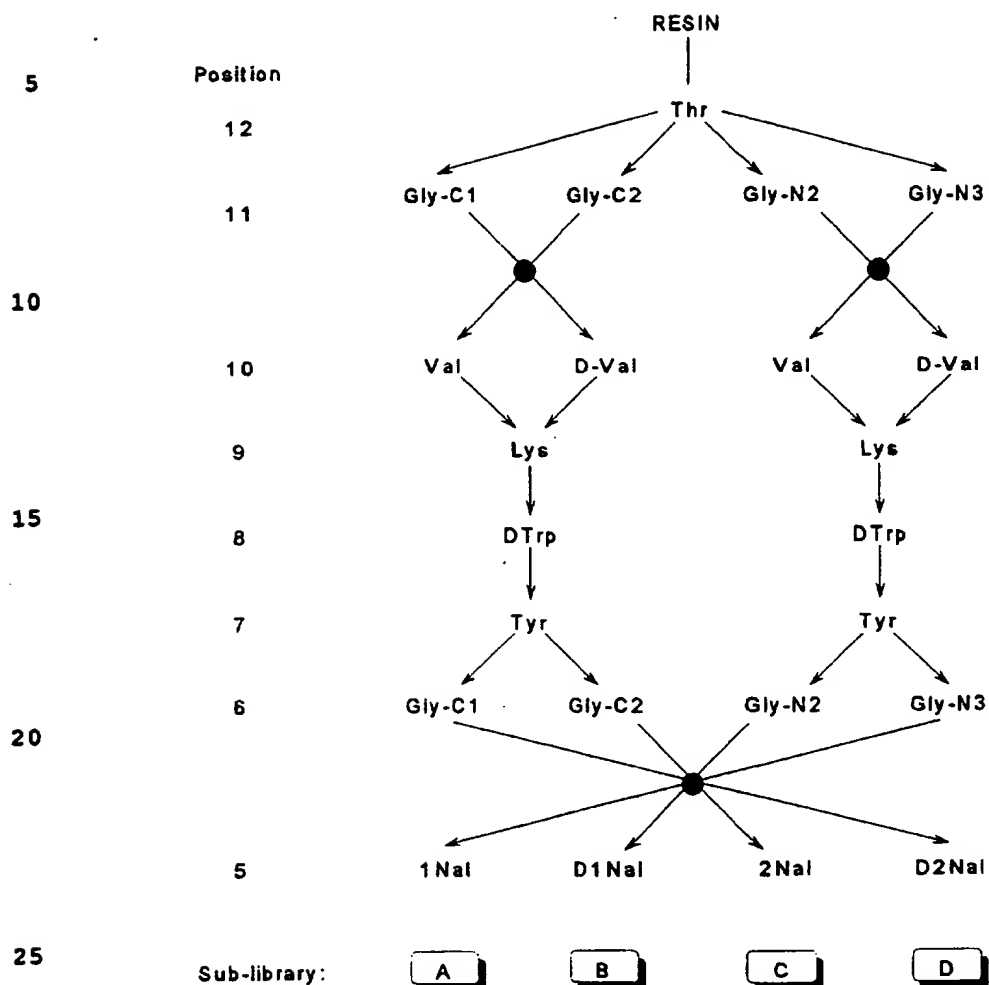
	A	B	C	D
hSSTR5	200	90	80	80
hSSTR4	400	400	400	400
mSSTR3			80	
mSSTR2				
hSSTR1	20	80	80	80

<u>YS5B LIBRARY</u>

Position						
12			RESIN			
			Thr			
11	Gly-C1	Gly-C2		Gly-N2	Gly-N3	
10	Val	D-Val		Val	D-Val	
9		Lys			Lys	
8		DTrp			DTrp	
7		Tyr			Tyr	
6	Gly-N2	Gly-N3		Gly-C1	Gly-C2	
5	D2Nal L2Nal	D2Nal L2Nal		D2Nal L2Nal	D2Nal L2Nal	

Sub-library: A   B   C   D
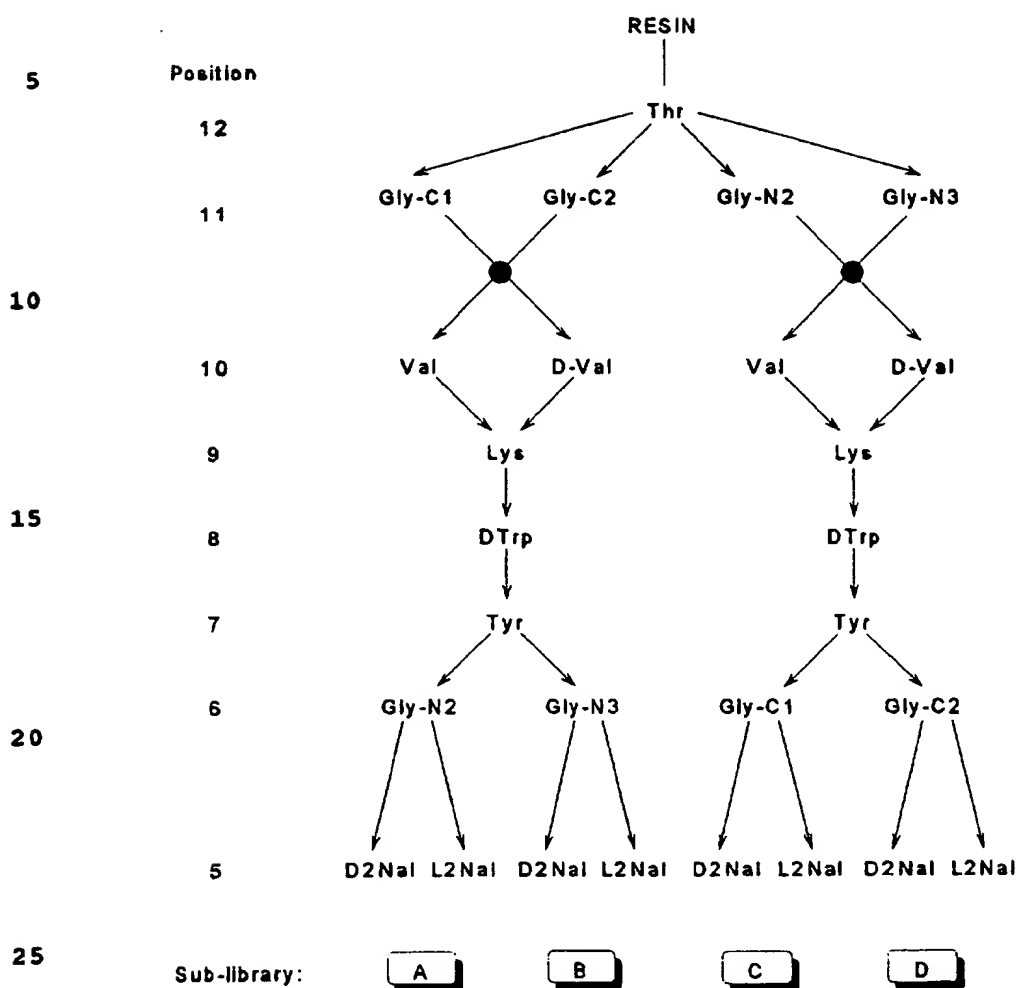
	A1	A2	B1	B2	C1	C2	D1	D2
hSSTR5	700	600	1000	1000	3000	3000	2000	1000
hSSTR4								
mSSTR3								
mSSTR2	3000	2000	1500	1500	1000	2500	1500	800
hSSTR1	400	400	400	400	1000	400	400	400

Example 75: VH-SST6 library

This library, which contains 24 hexapeptides, is described in table 14. Two different Phe-building units were incorporated at position R^6 with additional diversity at
5 positions R^7, R^8 and R^9. The peptides were cyclized between the backbone of positions R^6 and R^{10}. Amino acids at positions 5 and 12 were omitted.

Table 14. The composition of VH-SST6 library

Residues per position							
R^5	R^6	R^7	R^8	R^9	R^{10}	R^{11}	R^{12}
	Phe-C1 Phe-C2	Phe pNO$_2$Phe Phg	DTrp DThi	Lys Orn	Gly-C2	Phe	

15 **Example 76: VH-SST7 and VH-SST7A library**

These libraries each contain 290304 backbone-cyclic peptides in 45 sub-libraries. The peptides were synthesized on non-cleavable resin (TentaGel-NH2), yielding bead-attached peptides for screening in solid-phase-assays. The
20 composition of these libraries are descried in table VIII. The libraries differ from one another only in that VH-SST7 contains Trp at position 8, whereas VH-SST7A contains D-Trp at the same position.

25

30

35

Table 15.   Composition of VH-SST7 library.

Position	R^5	R^6	R^7	R^8	R^9	R^{10}	R^{11}	R^{12}	
Groups	A	B	C	D	E	F	G	H	
1	DPhe	Gly-N2	Phe	Trp or D-Trp	Lys	Thr	Gly-C1	Thr	
2	Phg	Gly-N3	Tyr	Tic	Arg	Val	Gly-C2	Ser	
3	1Nal	Phe-N2	Phg			Ser	Gly-C3	Val	
4	D1Nal	Phe-N3	pClPhe			Abu	Phe-C1	1Nal	
5	2Nal	Ala-N2	pFPhe				Phe-C2	D1Nal	
6	D2Nal	Ala-N3	pNO$_2$Phe				Phe-C3	2Nal	
7	Thi							D2Nal	
8	DThi								
9	pClPhe								
10	pFPhe								
11	pNO$_2$Phe								
12	Des								Total
Groups	12	6	6	2	2	4	6	7	45
Pept. per group	24192	12096	12096	145152	145152	72576	12096	41472	290304

The sub-libraries are named for their defined position:
A^1, A^2, ..A^n, B^1.. B^n, ...H^1 ..H^n.   For each group, positions
other than the defined one, contain mixture of amino acids.
In each coupling step, each non-defined position gets a
mixture of amino acids (total 1 molar equivalent of amino
acids in each step in order to force the completion of each
amino acid coupling and to eliminate kinetic effects,
yielding non-equal presentation of peptides), that will be
present at this position.   Identification of the most active
sub-library in each of the A to H group, by solid-phase
assay, will lead to the composition of most active backbone-

cyclic peptide from the 290304 peptides presented in the library.

Example 77: YS-SST6 library

5   This library comprises 128 backbone-cyclized somatostatin analogs in 8 sub-libraries described in table 16. Two basic cyclizations were used: position 3 to position 7 and position 2 to position 6. Each sub-library differs in the bridge location, bridge type and direction or amino acid

10   at position 1 (Ala or D2Nal).

Table 16. Structure of YS-SST6 sub-libraries.

Sub-library	Residues per position								
	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9
A1	Ala	Gly	Gly-C1 Gly-C2	Lys Arg	Asn DAsn	Phe	Gly-N2 Gly-N3	DTrp	Lys
A2	D2Nal	Gly	Gly-C1 Gly-C2	Lys Arg	Asn DAsn	Phe	Gly-N2 Gly-N3	DTrp	Lys
A3	Ala	Gly	Gly-N2 Gly-N3	Lys Arg	Asn DAsn	Phe	Gly-C1 Gly-C2	DTrp	Lys
A4	D2Nal	Gly	Gly-N2 Gly-N3	Lys Arg	Asn DAsn	Phe	Gly-C1 Gly-C2	DTrp	Lys
B1	Ala	Gly-C1 Gly-C2	Gly	Lys Arg	Asn DAsn	Gly-N2 Gly-N3	Phe	DTrp	Lys
B2	D2Nal	Gly-C1	Gly	Lys Arg	Asn DAsn	Gly-N2 Gly-N3	Phe	DTrp	Lys
B3	Ala	Gly-N2 Gly-N3	Gly	Lys Arg	Asn DAsn	Gly-C1 Gly-C2	Phe	DTrp	Lys
B4	D2Nal	Gly-N2 Gly-N3	Gly	Lys Arg	Asn DAsn	Gly-C1 Gly-C2	Phe	DTrp	Lys

Example 78: IG-SST9 library

       In order to more systematically test the necessity of

30 any given frame in the SRIF sequence for biological activity of the analogs, a library of octapeptide analogs that differ from one another in spanning different parts of the SRIF structure was synthesized in parallel. Each octapeptide sub-library is shifted from the next by one residue. Thus, the

35 first sub-library spands residues 7 to 14 the SRIF, the second sub-library spans residues 6 to 13 of SRIF and so on.

This library comprises a total of 14 overlapping backbone-
cyclized octapeptides with a shift of one residue between
each sub-library.

The synthesis is achieved by simultaneous synthesis of
5 the analogs from different starting points, such that the
coupling of the building units is performed for all the sub-
libraries at the same time. In all these sub-libraries the
backbone cyclization is accomplished between one Glycine-C2
unit distal to the N terminal of the peptide sequence and one
10 Glycine N3 unit proximal to the N terminal enc of the peptide
sequence.

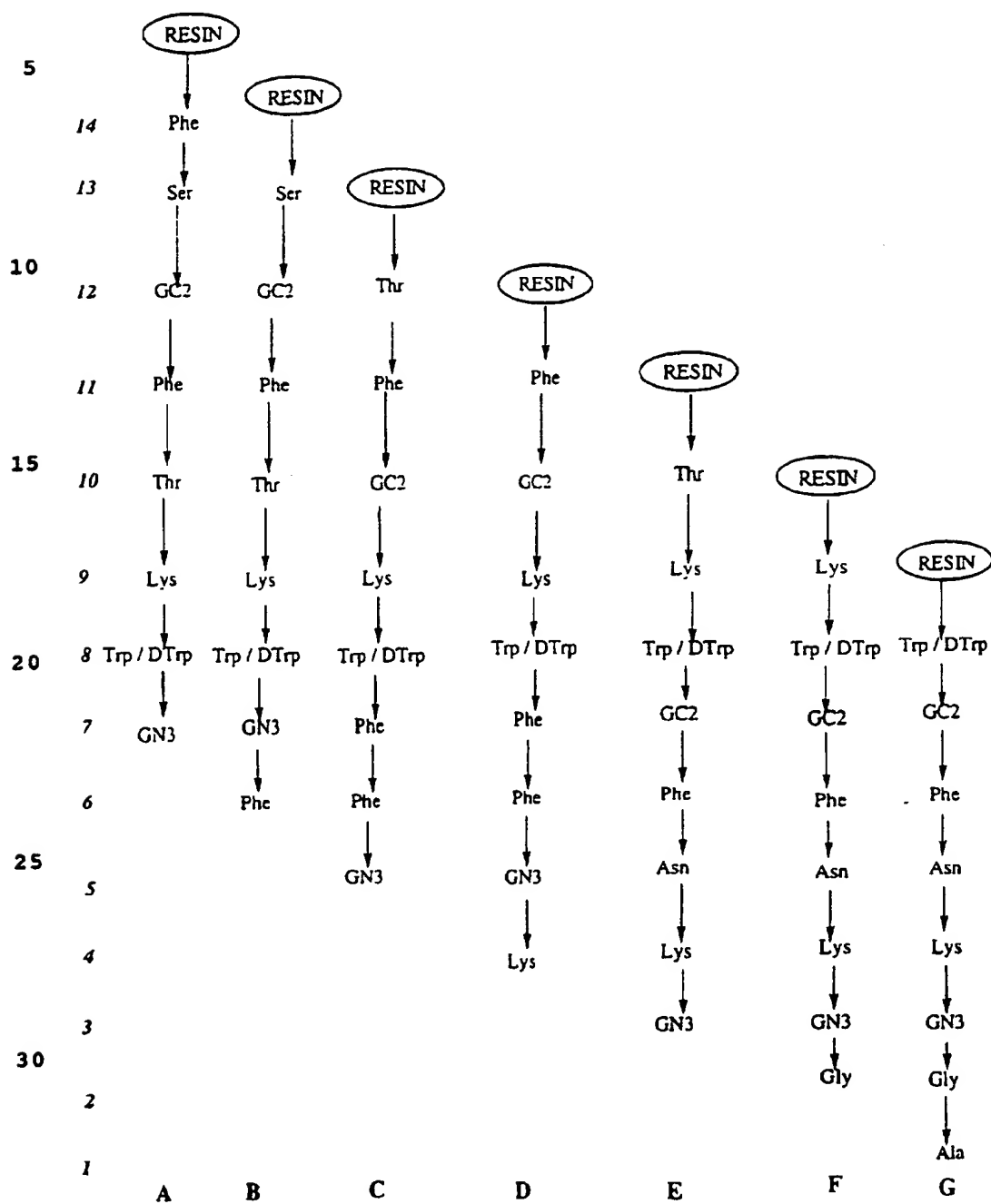Library IG-SST9 is represented in the following scheme:

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IG-SST9 Library

xample 79:   SST14 library

Different Phenylalanine-building units (PheBU: Phe-N2, Phe-N3, Phe-C2, Phe-C3), are used in this library as bridging arms for the generation of backbone cyclized analogs of SRIF

5 sequence 4-11 (sub-library D in IG-SST9 library).   In addition, the non bridging Phe residue (position 6 or 7), is substituted with various Phe and Nal derivatives: DPhe, pNO₂Phe, pClPhe, pFPhe, Phenylglycine (Phg), DPhg, L2Nal, D2Nal. This provides library of 18 groups and 16 analogs per

10 group as described in the following table:

Table 17. The composition of SST14 library

Residues per position							
R^4	R^5	R^6	R^7	R^8	R^9	R^{10}	R^{11}
Lys/DLys	Asn	Phe	Phe	Trp/DTrp	Lys	Thr	PheBu (4)
		PheBU (4)	PheBU (4)				
		DPhe	DPhe				
		pNO₂Phe	pNO₂Phe				
		pClPhe	pClPhe				
		pFPhe	pFPhe				
		Phg	Phg				
		DPhg	DPhg				
		L2Nal	L2Nal				
		D2Nal	D2Nal				

Example 80:   YS-SST7 library

The library contains 48 analogs with the following composition:

Table 18.   YS-SST7 library

Residues per position							
R^5	R^6	R^7	R^8	R^9	R^{10}	R^{11}	R^{12}
L2Nal	Gly-N3	pCl-Phe	DTrp	Lys	Ser	Gly-C2	Thr
DPhe		Tyr			Thr		L2Nal
					Val		D2Nal
					Abu		

Example 81. YS-SST10 library.

This library contains 5 sub-libraries with 24 peptides in
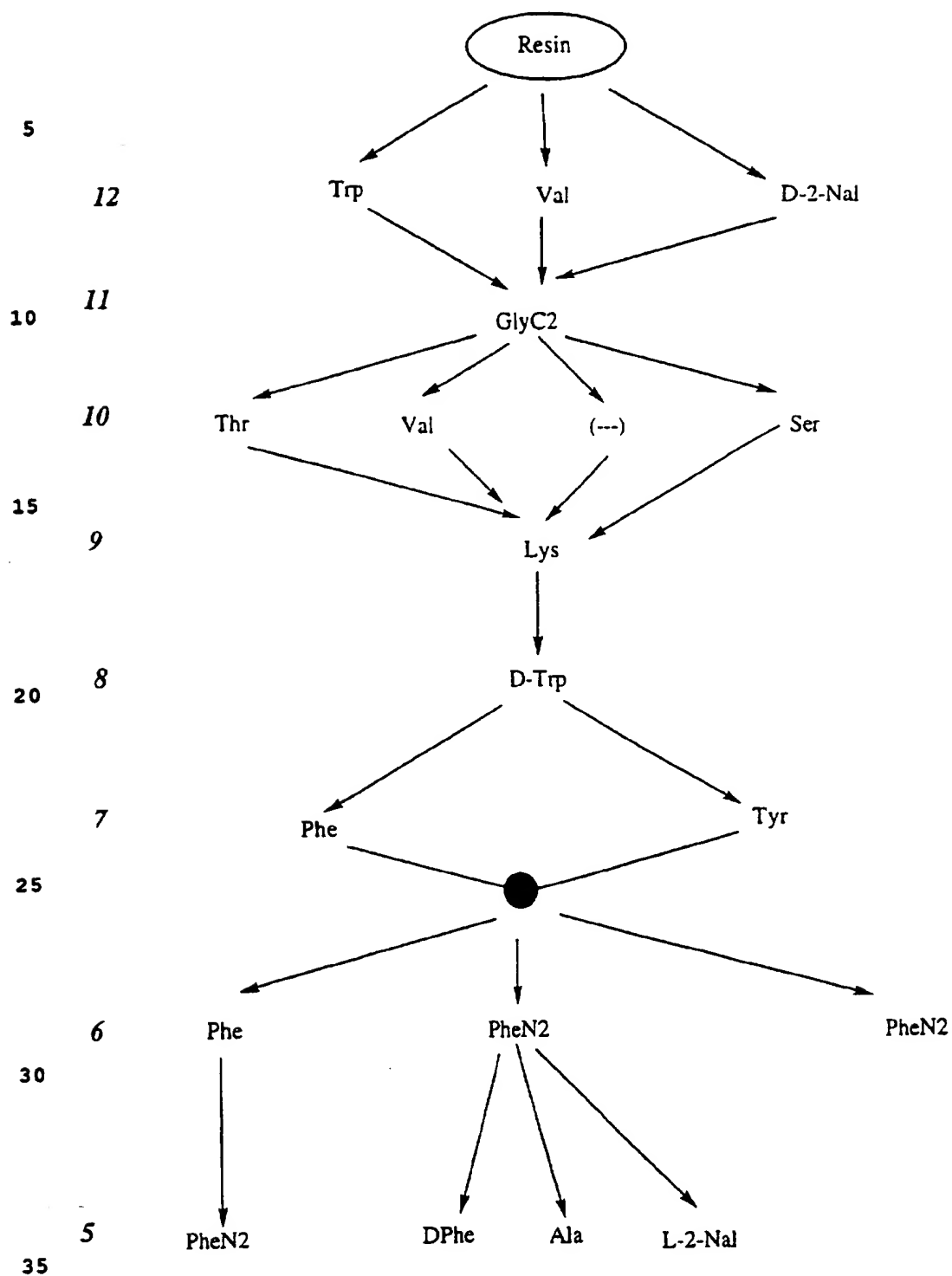each as described in the following scheme.

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Example 82. YS-SST12

The library, described in table 19, represents retro-backbone-cyclized analogs of somatostatin, and is composed of 6 sub-libraries, with 18 analogs in each. The different sub-libraries are defined by the type of bridge (Phe-C2^{11} to Gly-N3^{3} or Phe-N3^{11} to Gly-C2^{6}), and residue at position R^{10}.

Table 19. The composition of YS-SST12 library

Sub-library	Residues per position							
	R^{12}	R^{11}	R^{10}	R^{9}	R^{8}	R^{7}	R^{6}	R^{5}
A B C		Phe-C2	DVal DThr DGlu	DLys	Trp DTrp	DPhe pNH2Phep ClDPhe	Gly-N3	Phe L2Nal D2Nal
D E F		Phe-N3	DVal DThr DGlu	DLys	Trp DTrp	DPhe pNH2Phe pClDPhe	Gly-C2	Phe L2Nal D2Nal

Example 83. YS-SST15 library

This library of heptapeptides consist of 96 analogs in 8 sub-libraries defined by the residues at positions R^{6} and R^{7}.

Table 20. The composition of YS-SST15 library

Sub-library	Sequence per position							
	R^{5}	R^{6}	R^{7}	R^{8}	R^{9}	R^{10}	R^{11}	R^{12}
A B C D		Phe-N2 Phe-N3 Lys-N2 Gly-N2	Phe	DTrp Trp	Lys DLys	Gly	Phe-C3	Thr Val β-Ala
E F G H		Phe-N2 Phe-N3 Lys-N2 Gly-N2	Tyr	DTrp Trp	Lys DLys	Gly	Phe-C3	Thr Val β-Ala

PHYSIOLOGICAL EXAMPLES

Somatostatin analogs according to the present invention were tested for their activity in bioactivity assays *in vitro* and *in vivo*, in comparison to the following: the natural somatostatin peptide, i.e. SRIF; the known somatostatin

analog Octreotide; the non cyclized somatostatin derivatives, and/or irrelevant peptides as negative controls.

Example 84:   In vitro radio ligand binding assay for
5 **somatostatin.**

The somatostatin analogs were tested for their potency in inhibition of the binding of ^{125}I-Tyr^{11}-SRIF (based on the method described by Raynor et. al., Molecular Pharmacology 43, 838-844, 1993) to membrane preparations expressing the
10 transmembranal somatostatin receptors (SSTR-1,2,3,4 or 5). The receptor preparations used for these tests were either from the cloned receptors selectively and stably expressed in Chinese Hamster Ovary (CHO) cells or from cell lines naturally expressing the SSTRs. Typically, cell membranes
15 were homogenated in Tris buffer in the presence of protease inhibitors and incubated for 30-40 minutes with ^{125}I-Tyr^{11}-SRIF with different concentrations of the tested sample. The binding reactions were filtered, the filters were washed and the bound radioactivity was counted in gamma counter. Non
20 specific binding was defined as the radioactivity remaining bound in the presence of 1 μM unlabeled SRIF-14.
In order to validate positive signals of the binding tests, and to eliminate non-specific signals, samples of irrelevant peptides, such as GnRH, that were synthesized and handled
25 using the same procedures, were tested in the same assays as negative control samples. These samples had no binding activity in any of the assays.

Example 85:   Receptor binding specificity of cyclic peptide
30 **analogs.**

The various somatostatin receptor subtypes are thought to be involved in different signal transduction pathways. This will have implications in terms of choosing a somatostatin analog which shows specific and selective
35 binding to those receptor subtypes which are relevant to the disease that is to be treated. As reviewed by Reisine and

Bell (Endocrine Rev. 16, 427-442, 1995), the activities of several receptor subtypes is thought to be as follows:

SSTR-1 and SSTR-2: activation of tyrosine phosphatase which can lead to inhibition of EGF receptor
5 autophosphorylation, a process that is related to the antiproliferative effect of SST.

SSTR-2: inhibition of growth hormone and gastrin release, processes that are relevant to the treatment of acromegaly and anti proliferative effects via growth factors.

10   SSTR-5: inhibition of insulin, lipase, amylase release, activities that are relevant to the inhibition of calcium influx and to anti-proliferative effects of SST.

SSTR-3: involved in angiogenesis.

Binding of representative peptides of Examples 1-50 to
15 different somatostatin receptors was measured *in vitro*, in Chinese Hamster Ovary (CHO) cells expressing the various receptors. An example of the selectivity obtained with the cyclic peptides is presented in Table 21. The IC_{50} values presented are concentration required to inhibit 50% of
20 radioactive iodinated somatostatin (SRIF-14) binding.

Table 21 Estimated IC_{50} values (nM) for Inhibition of ^{125}I-SRIF-14 binding to cloned SSTRs by selected backbone-cyclized analogs.

PTR No.	SSTR-1	SSTR-2	SSTR-3	SSTR-4	human SSTR-5	rat SSTR-5
3004		> 10000	> 10000			> 2000
3007		> 10000	> 10000			> 2000
3010	2500	> 10000	> 1000	> 10000	> 2000	500
3013		> 10000	> 10000			> 2000
3016	2500		> 2000	> 2000	> 2000	1000
3019		> 10000	> 10000			
3022					> 2000	
3025			> 10000	> 2000	> 2000	> 2000
3034			> 10000	> 2000		> 2000
3040	100	> 1000	2500	1000	2000	100
3043	2000	2000	> 2000	1000	400	400
3046	3000	500	500	250	22	30

The IC_{50} values were calculated by testing the analogs at concentrations of 10^{-6}, 10^{-7}, 10^{-8} M in the radioligand binding assay described in example 84.

The affinities of PTR 3046 (synthetic example no. 42),
5 to the clones SSTRs are shown in figure 1. The unexpected advantages of PTR 3046 over other somatostatin analogs is in its selectivity. The analog binds with high affinity to the human SSTR-5 and much less to the other SSTRs.

Furthermore, the affinity to the rat and the human SSTR5
10 are similar for PTR 3046, thus, drug dosages given in rat models may predict the efficacy in humans. For the sake of comparison the affinities of PTR 3046 are presented in Table 22 together with those obtained for known SST analogs.

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Table 22. Selectivity of PTR 3046 versus known SST analogs to
SSTR subtypes.

Analog	IC$_{50}$ value (nM)					
	SSTR 1	SSTR 2	SSTR 3	SSTR 4	hSSTR 5	rSSTR 5
SRIF-14*	0.1	0.3	0.1	1.2	0.2	0.3
Octreotide*	>1000	0.4	150	>1000	32	0.2
Somatuline (BIM-23014)*	800	2	6	>1000	14	0.5
RC-160**	200	0.2	0.1	620	21	0.2
BIM-23052*	23	32	0.4	18	4	0.004
PTR 3046	3000	500	500	250	22	30

* Reisine and Bell (1995), Endocrine Reviews 16; 427-442.
** L. Buscail et. al. (1995), PNAS 92; 1580-1584.

Another cyclic peptide analog, PTR 3040, showed an
interesting profile of selectivity. This analog shows
relatively high affinity for receptor subtype SSTR-1, and
very low affinity for the other receptor subtypes. While PTR
3040 showed high binding to the rat SSTR-5, its affinity to
the cloned human receptor was significantly lower.

Inhibition of ^{125}I-SRIF binding to mouse pituitary AtT20
cells was also tested for various analogs. The results
obtained with PTR 3046 and PTR 3040 are presented in Figure
2, compared to SRIF-14. The results represent the percent
inhibition per specific concentration of each of the
compounds.

**Example 86: Receptor binding specificity of libraries of
cyclic peptide analogs.**

Binding of representative sub-libraries of peptides of
Examples 68-83 to different somatostatin receptors was
measured in vitro, in Chinese Hamster Ovary (CHO) cells
expressing the various receptors. An example of the
selectivity obtained with the cyclic peptide libraries is

presented in Table 23. The values presented are percent
inhibition of radioactive iodinated somatostatin (SRIF-14)
binding.

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Table 23. Estimated IC$_{50}$ values (nM) for Inhibition of ^{125}I-SRIF-14 binding to cloned SSTRs by selected backbone-cyclized sub-libraries.

Sub-library	Pep. *	SSTR-1	SSTR-2	SSTR-3	SSTR-4	human SSTR-5	rat SSTR-5
VH1-A	8						8000
VH1-B	8						6400
VH1-C	8						7200
VH1-D	8						1600
VH1-E	8	1600		>2000	8000	6400	640
YS1-A1-2	4				>5000		3200
YS1-A3-4	4				>5000		800
YS1-B1-2	4	360	6000	3600	600	1600	200
YS1-B1-4	4				>5000		8000
YS2-A	12				3600		100
YS2-B	12				3600		120
YS2-C	12	110	1200	300	1000	100	25
YS2-D	12	960	300	4800	1800	3000	25
YS4	24	3500			2400	2400	2000
VH6	24						7200
YS5A-A	16	320			6400	3200	1300
YS5A-B	16	1300			6400	1400	
YS5A-C	16	1300		1300	6400	1300	
YS5A-D	16	1300			6400	1300	
YS5B-A1	4	400	3000			700	
YS5B-A2	4	400	2000			600	
YS5B-B1	4	400	1500			1000	
YS5B-B2	4	400	1500			1000	
YS5B-C1	4	1000	1000			3000	
YS5B-C2	4	400	2500			3000	
YS5B-D1	4	400	1500			2000	
YS5B-D2	4	400	800			1000	

* Number of different peptides in each sub-library.

The estimated IC$_{50}$ are calculated based on the concentration of total peptides in each sub-library mixture. However, each sub-library is composed of different peptides that might have different activity. The activity of the best peptide in any given sub-library might therefore be higher (i.e., lower IC$_{50}$ value), to the extent of the given value divided by the number of peptides per sub-library. In addition, the activity values of purified individual peptides might be better due to existence of salts and impurities in the total weight of the sub-library used for calculation of the concentration.

In vivo examples:

Selected analogs were tested in-vivo, for the inhibition of growth-hormone release, amylase, lipase, gastrin, insulin, cholecystokinin (CCK), VIP and glucagon secretion in animals.
5 Growth hormone (GH) release is related to acromegaly. Gastrin secretion is related to gastrinoma (Zollinger-ellison syndrome) and ulcer. Insulin release is related to insulinoma, hyperinsulinoma, obesity and NIDDM. Glucagon release is related to hyperglycemia, NIDDM and glucagonemia.
10 Amylase and lipase release are related to acute and chronic pancreatitis, enterocutaneous and pancreatic fistulas and pancreatic surgery. VIP release is related to VIPoma and secretory diarrhea. In addition, the antiproliferative effects of somatostatin can be direct or indirect through GH-
15 IGF or CCK release.

Example 87: Somatostatin biological activity assays (*In vivo* **assays.**

The *in vivo* biological effects of SST analogs on growth
20 hormone, insulin and glucagon release is tested by measuring the levels of these hormones using commercially available RIA test kits. Pharmacological effects of SST in patients with neuroendocrine tumors of the gut will require determination of 5-hydroxyindole acetic acid (for carcinoid tumors) and VIP
25 (for VIPoma). *In vivo* visualization of SST receptor-positive tumors is performed as described by Lambert el al. (New England J. Med., 323:1246-1249 1990), following i.v. administration of radio-iodinated SST analogs.

30 Example 88: Resistance to biodegradation of SST analogs.

The *in vitro* biostability of a SST cyclic peptide analog, PTR 3002, was measured in human serum, and was compared to the same sequence in a non-cyclic peptide analog (PTR 3001), to Octreotide (Sandostatin™), and to native
35 somatostatin (SRIF). In this assay, the cyclic peptide in accordance with the present invention is as stable as Octreotide, is more stable than the corresponding non-cyclic

structure, and is much more stable than SRIF. The assay was
based on HPLC determination of peptide degradation as a
function of time in serum at 37°C.

5 **Example 89: Inhibition of growth hormone release by SST
analogs.**

        In vivo determination of the pharmacodynamic properties
of cyclic peptide SST analogs was carried out in rats,
according to known procedures. Inhibition of Growth Hormone
10 (GH) release as a result of peptide administration was
measured in Sprague-Dawley male rats. The SST cyclic
peptide analog activity was compared in this study to SRIF or
to Octreotide using 4 rats in each group. Time course
profiles for GH release under constant experimental
15 conditions were measured.

Methods

        Adult male Sprague-Dawley rats, specific pathogen free
(SPF), weighing 200-350 g, were maintained on a constant
light-dark cycle (light from 8:00 to 20:00 h), temperature
20 (21 ± 3°C), and relative humidity (55 ± 10%). Laboratory chow
and tap water were available ad libitum. On the day of the
experiment, rats were anesthetized with pentobarbitone
(50 mg/kg). Rats anesthetized with pentobarbitone exhibit
low somatostatis levels in portal blood vessels (Plotsky,
25 P.M., Science, 230, 461-463, 1985). A single blood sample
(0.6 ml) was taken from the exposed cannulated jugular vein
for the determination of the basal GH levels (-15 min.).
Immediately thereafter the appropriate peptide treatment was
administered. The animals received 10 mg/kg of either native
30 somatostatin (SRIF), the synthetic analog Octreotide
(Sandostatin), or the cyclic peptide analog. A physiological
saline solution (0.9% NaCl) was administered as a control.
All peptides were administered subcutaneously in a final
volume of 0.2 ml. Further sampling was carried out at 15,
35 30, 60, and 90 minutes after peptide administration. Blood
samples were collected into tubes containing heparin (15

units per ml of blood) and centrifuged immediately. Plasma
was separated and kept frozen at -20°C until assayed.

Rat growth hormone (rGH) [^{125}I] levels were determined by
means of a radioimmunoassay kit (Amersham). The standard in
5 this kit has been calibrated against a reference standard
preparation (NIH-RP2) obtained from the National Institute of
Diabetes and Digestive and Kidney Diseases. All samples were
measured in duplicate.

The results of these experiments show that Octreotide
10 significantly inhibits the release of growth hormone whereas
the cyclic analog PTR 3046, that does not bind the SSTR-2, is
not significantly different from saline in this test, as
expected for a somatostatin analog which is selective for the
SSTR-5 receptor subtype.
15   The data of these studies are summarized in Figure 3.

Example 90: Lack of toxicity of cyclized peptide analogs.

PTR 3007 at a dose of 1.5 mg/kg was well tolerated after
single intraperitoneal application. PTR 3013 was not toxic
20 to the rats even with doses of 4 mg/kg. These two doses are
several orders of magnitude higher than those needed to
elicit the desired endocrine effect. The peptides dissolved
in saline produced no untoward side effects on the central
nervous system, cardiovascular system, body temperature, nor
25 on the periphery of the animals. Rats were observed for
4 hours post administration of the peptides. PTR 3007 and
3013 produced no respiratory disturbances, did not result in
the appearance of stereotyped behavior, or produce any
changes in muscle tone. After 3 hours, postmortem
30 examination did not detect any abnormality in the liver,
kidneys, arteries and veins, gastrointestinal tract, lungs,
genital system, nor the spleen.

Example 91. The *in vivo* effect of somatostatin analogs on
35 **glucose induced insulin release in rats.**

Based on the known physiology of the native somatostatin
as an inhibitor of insulin release, the aim of this study was

- 74 -

to evaluate the effect of the backbone cyclic analog PTR 3046
on the postprandial secretion of insulin.

Experimental:

5   Adult male Wister rats, specific pathogen free (SPF) weighing
    200-220 g were used. The animals were maintained on a
    constant light- dark cycle (light from 8.00 to 20.00 h),
    Temp. (21°C), and humidity (55%). The animals were allowed
    free access to food and water until 18-20 hours before the
10 experiment, when all food (but not water) was withdrawn. The
    animals were housed in plastic cages with wide mesh wire
    bottoms to prevent coprophagia (feeding on excrement). On
    the day of the experiment, rats were anesthetized with
    pentobarbitone (60 mg/kg IP). 15 minutes after pentobarbital
15 administration a catheter was inserted into the right
    external jugular vein to allow sampling of blood. Body
    temperature was monitored using a rectal temperature digital
    thermometer, and maintained at a constant level (37-37.5∞C)
    by heated blanket placed beneath the rat in addition to two
20 100W bulbs illuminating the operation table from a distance
    of 50 cm. After cannulation, a 0.7 ml sample of blood was
    drawn from the jugular vein, and transferred into tubes into
    which 20 IU heparin solution 5000 IU/ml was previously
    prepared. This blood sample was collected for the
25 determination of the basal insulin levels. Immediately
    thereafter the appropriate peptide pre-treatment was
    administered.

        The animals received the following peptides: The
    synthetic analog Octreotide at 10 μg/kg (n=15), the backbone
30 cyclic peptide PTR 3046 at 7 μg/kg (n=18). Saline (0.9%
    NaCl) 0.2 ml was administrated as a control (n=16). All
    peptides were administered subcutaneously in a final volume
    of 0.2 ml. 10 minutes after drug administration the next
    blood sample was drawn, immediately after that a glucose
35 solution was administrated IV at a final dose of 0.5 g/kg.

Further sampling was carried out at 2 and 5 min. post glucose
administration.

    Immediately after the collection of each blood sample an
appropriate volume (0.7 ml) of saline was administered IV.
5 Blood samples were collected into tubes containing heparin
(15 units per ml of a blood) and centrifuged (1500 g)
immediately and plasma separated and kept frozen at -20∞C
(until assayed).

    Rat insulin (rIns) [^{125}I] levels were determined by means
10 of rat insulin RIA kit. This kit utilizes an antibody made
specifically against rat insulin (Linco). The sensitivity of
the kit was 0.1 ng/ml. All samples were measured in
duplicate.

15 <u>Results</u>:

    Administration of 7 μg/kg of PTR 3046 led to a
significant ($p < 0.05$) decrease (43%) in insulin release in
comparison to untreated control rats. The decrease of the
insulin levels in Octreotide pretreated rats were not
20 statistically significant in this experiment.

    These results demonstrated that the backbone cyclic
peptide PTR 3046 is a potent inhibitor of postprandial
insulin release in vivo. Based on the present study and the
manufacturer's data reported for Octreotide (ED 50 for
25 insulin release of 26 μg/kg) it is concluded that in vivo PTR
3046 is 4 times more potent than Octreotide on insulin
release.

    Hyperinsulinemia is one of the etiologic factors
associated with the pathology of obesity and the early stage
30 of noninsulin dependent diabetes mellitus (NIDDM).
Therefore, the potential application of PTR 3046 as an anti
secretagogue on insulin release should be considered in view
of the high inhibition of insulin secretion with this novel
SST analog.
35   The data of these studies are summarized in Figure 4.

Example 92:   Effect of somatostatin analogs on bombesin stimulated plasma amylase and lipase release.

    Inhibition of either insulin or amylase release has been
reported (Reisine and Bell, ibid.) to be highly correlated
5 with affinity to the SST receptor subtype SSTR-5, while i
inhibition of pituitary growth hormone (GH) release,
pancreatic glucagon, and gastric acid secretion were SSTR-2
mediated. Binding affinity data presented above (Example
85), demonstrated the selectivity of the cyclic heptapeptide
10 PTR 3046 to hSSTR-5 (affinity 20 nM) in comparison to its low
affinity (>1000 nM) to SSTR-1, 2, 3 and 4. Based on these
results the preliminary evaluation of the physiological
activity of PTR 3046 was evaluated using the *in vivo* model of
bombesin stimulated lipase and amylase release. The dose
15 response of PTR 3046 in terms of inhibition of amylase and
lipase release in rats was measured following administration
of the peptide analog at three different dosages i.e., 3
μg/kg, 12.5 μg/kg and 25 μg/kg. These doses were compared to
the synthetic analog SMS-201 995 administered at a dose of 10
20 μg/kg.

Methods

    Male Wister rats (weighing 200-220 g) were anaesthetized
with pentobarbital (60 mg/kg) by the intraperitoneal (IP)
25 route. The animals were allowed free access to food and
water until 18 h before experiment, when all food (but not
the water) was withdrawn. Furthermore the animals were
housed in cages with wide mesh wire bottoms to prevent
coprophagia (feeding on excrement).
30

Collection of samples

    After cannulation, a 0.7 ml sample of blood was drawn
from the jugular vein, and transferred into an Eppendorf tube
containing 20 IU heparin solution 5000 IU/ml. One minute
35 later the peptides were administered subcutaneously (SC in
0.2 ml of 0.9% NaCl). Control rats were pretreated with

0.2 ml of 0.9% NaCl. At time 0 (5 min. after collection of
baseline blood sample-1) a bombesin infusion (50 nmol/kg/hr)
was started in all animals. Additional blood samples were
collected at constant time intervals of 60, 90 and 120 min.
5 during bombesin infusion.

Treatment of samples

     Blood samples were collected into ice-cold tubes
containing heparin (20 IU/ml) and were centrifuged (1500 g x
10 10 min.). Plasma samples were frozen and kept at -20°C until
assayed for amylase and lipase.

Analytical- assays

Amylase levels were measured in plasma with the commercial
15 (Raichem™) Amylase reagent.

Lipase levels were measured in plasma with a commercial kit
(Randox™).

20 <u>Results</u>
<u>Prevention of bombesin- stimulated amylase secretion by SST</u>
<u>analogs</u>:

Control (saline pretreatment):
25   Bombesin I.V. infusion at a dose of 50 nmol/kg/h
resulted in a time dependent increase of plasma amylase (by
10 fold above basal at 60 and 90 min.), and lipase (by 14
fold above basal at 60 and 90 min.).

30 PTR 3046:
     Pretreatment with PTR 3046 resulted in significant dose
dependent inhibition of bombesin induced release of plasma
amylase in comparison to control rats. Inhibition was 31% at
90 min. and 23% at 120 min. for 3 μg/kg, and 60% at 90 min.
35 and 52% at 120 min. for the dose of 25 μg/kg. Pretreatment

with Octreotide at 10 μg/kg resulted in a significant
inhibition 23% at these time points.

Prevention of bombesin-stimulated lipase secretion by SST
5 analogs:

PTR 3046:
        Pretreatment with PTR 3046 resulted in significant dose
dependent inhibition of bombesin induced release of plasma
10 amylase in comparison to control rats. Inhibition was 33% at
90 min. and 26% at 120 min. for 3 μg/kg, 30% at 90 min. and
25% at 120 min. for 12.5 μg/kg, and 51% at 90 and 35% at
120 min. for the dose of 25 μg/kg. While pretreatment with
SMS at 10 μg/kg resulted in a significant inhibition of 27%
15 and 30% at 90 and 120 min. respectively.
        Similar results were obtained with PTR 3010 (data not
shown).
        These results demonstrate that PTR 3046 is a selective
analog for SSTR-5 as shown in preliminary binding assays and
20 physiological model of enzymes and endocrine secretion.
        Bombesin induced pancreatic and gastric secretion is a
common in vivo model for the evaluation of anti secretory
effect of bombesin antagonist, bombesin antibodies, and
somatostatin analogs.
25
        The putative role of bombesin in several lung and gut
diseases in humans suggest that drugs that inhibit the
secretory effect of bombesin, like PTR 3010, and PTR 3046
could be used as pharmacotherapy for various therapeutic
targets associated with bombesin, including: secretory
30 diseases such as pancreatitis, rhinitis and gastrinoma;
neurocrine cell hyperplasia diseases such as bronchopulmonary
dysplasia, cystic fibrosis, and chronic bronchitis and
emphysema; proliferative diseases such as prostate
hypertrophy, prostate cancer, pancreatic cancer and stomach
35 cancer.

Furthermore, portal hypertension, GI bleeding, and colorectal carcinoma could be potential applications as well.

Example 93. Duodenal-pancreatic Perfusion

5 A limitation with the Bombesin or the Caerulein models was that, both tests were based on the indirect detection of levels of enzymes in the serum. Because amylase can be released from saliva and other sites in the GI tract, there was a need for a direct measurement of effect on
10 the pancreatic release of enzymes. To that end, the perfusion model was developed in the rat. The results (Fig. 5) indicate that PTR 3046 and Sandostatin (administrated by IV infusion), both inhibit the pancreatic release of enzymes, as detected directly in the duodenal
15 perfusate.

The duodenal segment which drains the pancreatic release was dissociated proximally from the stomach and distally from the jejunum. The segment was perfused with physio-
20 logical saline, perfusate was collected at 15 min. time intervals. Stimulation of pancreatic release was done by IV infusion of Bombesin or Caerulein, 1 nMole/kg/h or 4 nMole/kg/h respectively. During perfusion (after the enzyme levels reached plateau levels) drugs were infused
25 IV for another 2 h. Pancreatic enzyme levels were measured in the perfusate samples. Data are presented as percentage of average enzyme levels from bombesin induced plateau levels.

30

Example 94. Antiproliferative activity

Based on the inhibition of exocrine release induced by caerulein (a CCK analog), and because CCK is a potent
35 grow factor in the GI tract, it was rational to test the potential anti proliferative activity of PTR 3046 on cancer cell lines derived from GI tumors.

A significant antiproliferative effect of PTR 3046 was
found on the human pancreatic cell line, MiaPaca-2 (Fig-
ure 6).

Cells were grown in DMEM culture media supplemented with
5 10% FCS. Cells allowed to attached after 24 h to the
plate. Drugs were added to the culture media in concen-
tration range of 10^{-5} M-10^{-11} M. Proliferation of cells was
evaluated by MTT assay at 24, 48, and 72 hours following
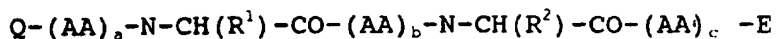drug application.

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THE CLAIMS

What is claimed is:

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1. A backbone cyclized somatostatin analog having the general Formula (I):

$$Q-(AA)_a-N-CH(R^1)-CO-(AA)_b-N-CH(R^2)-CO-(AA)_c-E$$

10

Formula (I)

wherein: a - c each independently designates an integer
15 from 1 to 8 or zero; (AA) designates an amino acid residue wherein the amino acid residues in each chain may be the same or different; Q represents H or an acyl group; E represents a hydroxyl group, a carboxyl protecting group or an amino group, or the terminal carboxyl
20 group can be reduced to CH_2-OH; R^1 and R^2 each designates an amino acid side-chain optionally bound with a specific protecting group; and the line designates a bridging group of the Formula: $-(CH_2)_x-M-(CH_2)_y-$; M is selected from the group consisting of amide, thioether, thioester and
25 disulfide; and x and y each independently designates an integer from 1 to 10.

2. The backbone cyclized somatostatin analog of Claim 1
30 having the general Formula (Va):

$$R^5-NR^6-R^7-(D)-Trp-Lys-R^{10}-NR^{11}-R^{12}-X$$

$$(CH_2)_m-Y^2-(CH_2)_n$$
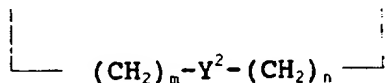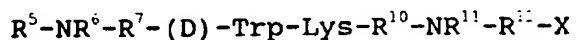
35   Formula (Va)

wherein m and n are 1 to 5; X designates a carboxy terminal amide or alcohol; R^5 is absent or is Gly, (D)- or (L)- Ala, Phe, Nal and β-Asp(Ind); R^6 and R^{11} are independently Gly or (D)- or (L)-Phe; R^7 is Phe or Tyr; R^{10} is absent or is Gly, Abu, Thr or Val; R^{12} is absent or is Val, Thr or Nal, and Y^2 is selected from the group consisting of amide, thioether, thioester and disulfide.

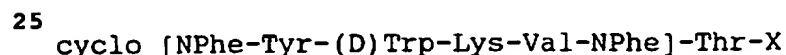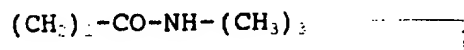3.   The backbone cyclized peptide analog of Claim 2 having the general Formula (Vb):

$$H-NR^6-R^7-(D)Trp-Lys-R^{10}-NR^{11}-Thr-X$$

$$\llcorner (CH_2)_m-Y^2-(CH_2)_n \lrcorner$$
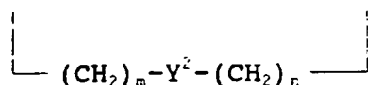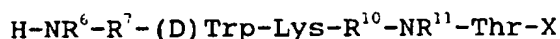
Figure (Vb)

wherein m and n are 1 to 5; X designates a carboxy terminal amide or alcohol; R^6 and R^{11} are independently Gly or (D)- or (L)-Phe; R^7 is Phe or Tyr; R^{10} is absent or is Gly, Abu, Thr or Val; and Y^2 is selected from the group consisting of amide, thioether, thioester or disulfide.

4.   The backbone cyclized somatostatin analog of claim 3 having the structure:
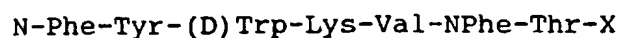
cyclo [NPhe-Tyr-(D)Trp-Lys-Val-NPhe]-Thr-X

wherein X is denotes a carboxy terminal acid, amide, ester or alcohol.

5.   The backbone cyclized somatostatin analog of claim 4 having the structure

$(CH_2)_2-CO-NH-(CH_3)_2$

N-Phe-Tyr-(D)Trp-Lys-Val-NPhe-Thr-X

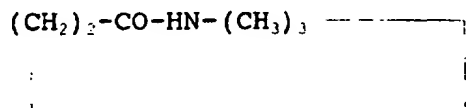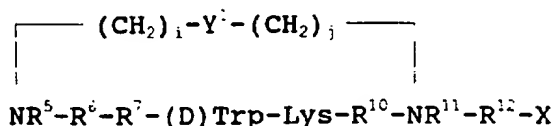wherein X denotes a carboxy terminal acid, amide, ester or alcohol.

6.   The backbone cyclized somatostatin analog of claim 3 having the structure:

cyclo [NPhe-Phe-(D)Trp-Lys-Thr-NPhe]-Val-X

wherein X denotes a carboxy terminal acid, amide, ester or alcohol.

7.   The backbone cyclized somatostatin analog of claim 6 having the structure

$$(CH_2)_2-CO-HN-(CH_3)_3 \text{ -- -- -------}$$

N-Phe-Phe-(D)Trp-Lys-Thr-NPhe-Val-X

wherein X denotes a carboxy terminal acid, amide, ester or alcohol.

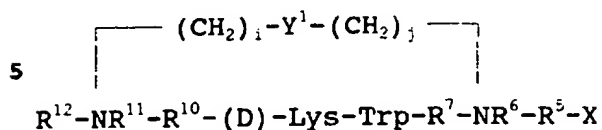8.   The backbone cyclized somatostatin analog of Claim 1 having the general Formula (XI):

Formula XI

$$\text{---}(CH_2)_i-Y^1-(CH_2)_j\text{---}$$

$$NR^5-R^6-R^7-(D)Trp-Lys-R^{10}-NR^{11}-R^{12}-X$$

wherein i and j are independently 1 to 5; X designates a carboxy terminal amide or alcohol; R^5 is (D)Phe or (L)Phe, Ala or Lys; R^6 is absent or is Phe; R^7 is Tyr or Phe; R^{10} is absent or Thr, Val, Ser or Abu, R^{11} is Phe, Gly or Ala; R^{12} is Trp, Thr, Val, 2-Nal or (D)2-Nal, and Y^1 is selected from the group consisting of amide, thioether, thioester and disulfide.
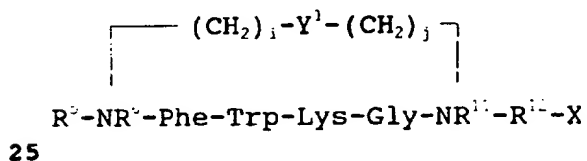
- 84 -

9.   The backbone cyclized somatostatin analog of Claim 1
having the general Formula (XII):

$$\begin{array}{c} \text{┌─── (CH}_2\text{)}_i\text{-Y}^1\text{-(CH}_2\text{)}_j \text{───┐} \\ | \qquad\qquad\qquad\qquad\qquad | \\ R^{12}\text{-NR}^{11}\text{-R}^{10}\text{-(D)-Lys-Trp-R}^7\text{-NR}^6\text{-R}^5\text{-X} \end{array}$$

Formula XII

wherein i and j are independently 1 to 5; X designates a
carboxy terminal amide or alcohol; R^5 is Phe,
(L)2-Naphtylalanine or (D)2-Naphtylalanine; R^6 is Phe, Gly
or Ala; R^7 is (D)Phe, pCl(D)Phe, pNH$_2$Phe or (D)Tyr; R^{10} is
(D)Thr, (D)Val, (D)Ala, (D)Leu or (D)Glu; R^{11} is Phe, Gly
or Ala; R^{12} is absent or is Thr or Val, and Y^1 is selected
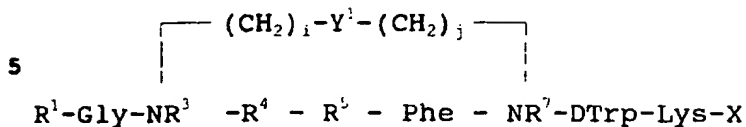from the group consisting of amide, thioether, thioester
and disulfide.

10.   The backbone cyclized somatostatin analog of Claim 1
having the general Formula (XIII):

$$\begin{array}{c} \text{┌─── (CH}_2\text{)}_i\text{-Y}^1\text{-(CH}_2\text{)}_j \text{─┐} \\ | \qquad\qquad\qquad\qquad\qquad | \\ R^5\text{-NR}^6\text{-Phe-Trp-Lys-Gly-NR}^{11}\text{-R}^{12}\text{-X} \end{array}$$

Formula XIII

wherein i and j are independently 1 to 5; X designates a
carboxy terminal amide or alcohol; R^5 is absent or (D)Phe
or 2-Nal; R^6 is Phe, Gly, Lys or Ala; R^7 is (D)Phe,
pCl(D)Phe, pNH$_2$Phe or (D)Tyr; R^{10} is (D)Thr, (D)Val,
(D)Ala, (D)Leu or (D)Glu; R^{11} is Phe, Gly or Ala; R^{12} is
Thr, Val, Ala, β-Ala, (L)2-Naphtylalanine or (D)2-
Naphtylalanine, and Y^1 is selected from the group
consisting of amide, thioether, thioester and disulfide.

11.   The backbone cyclized somatostatin analog of Claim 1
having the general Formula (XIV):

$$\begin{array}{c} \overline{}(CH_2)_i-Y^1-(CH_2)_j\overline{} \\[4pt] | \qquad\qquad\qquad\qquad | \\[4pt] R^1\text{-Gly-NR}^3 \quad -R^4 \ - \ R^5 \ - \ Phe \ - \ NR^7\text{-DTrp-Lys-X} \end{array}$$

Formula XIV

wherein i and j are independently 1 to 5; X designates a
carboxy terminal amide or alcohol; R^1 is Ala or (D)2-
Naphtylalanine; R^4 is Lys or Arg; R^5 is (L)Asn or (D)Asn;
R^7 is Phe, Gly, Ala or Lys, and Y^1 is selected from the
group consisting of amide, thioether, thioester and
disulfide.

12.   A   pharmaceutical   composition   comprising   the
somatostatin   analog   of   any   one   of   claims   1-11   and   a
pharmaceutically acceptable carrier or dilutent.

13.   The pharmaceutical composition of claim 12 wherein
the somatostatin analog is
cyclo [NPhe-Tyr-(D)Trp-Lys-Val-NPhe]-Thr-X   wherein   X   is
denotes a carboxy terminal acid, amide, ester or alcohol.

14.   The pharmaceutical composition of claim 13 in unit
dosage form.

15.   The pharmaceutical composition of claim 12 wherein
the somatostatin analog is
   cyclo [NPhe-Phe-(D)Trp-Lys-Thr-NPhe]-Val-X
wherein X denotes a carboxy terminal acid, amide, ester
or alcohol.

16.   The pharmaceutical composition of claim 15 in unit
dosage form.

17. A method of treating endocrine disorders, neoplasms
or metabolic disorders which comprises administering to
an individual in need thereof a therapeutically effective
amount of the somatostatin analog of any one of claims 1-
5 11.

18. Use of the somatostatin analog of any one of claims
1-11 in the manufacture of a medicament for the treatment
of endocrine disorders, neoplasms or metabolic disorders.
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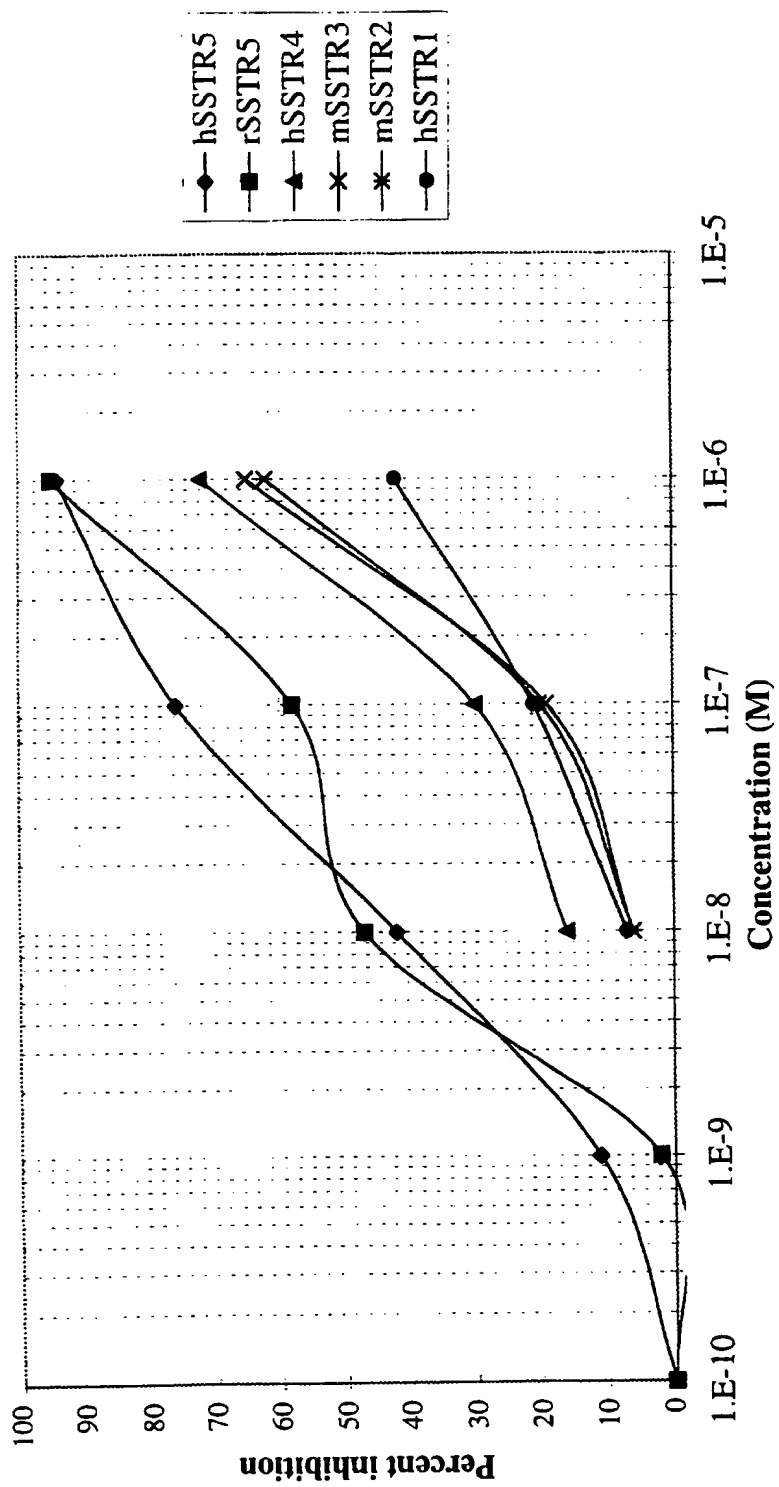35

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Figure 1. In-vitro inhibition of SRIF binding
to cloned SSTRs

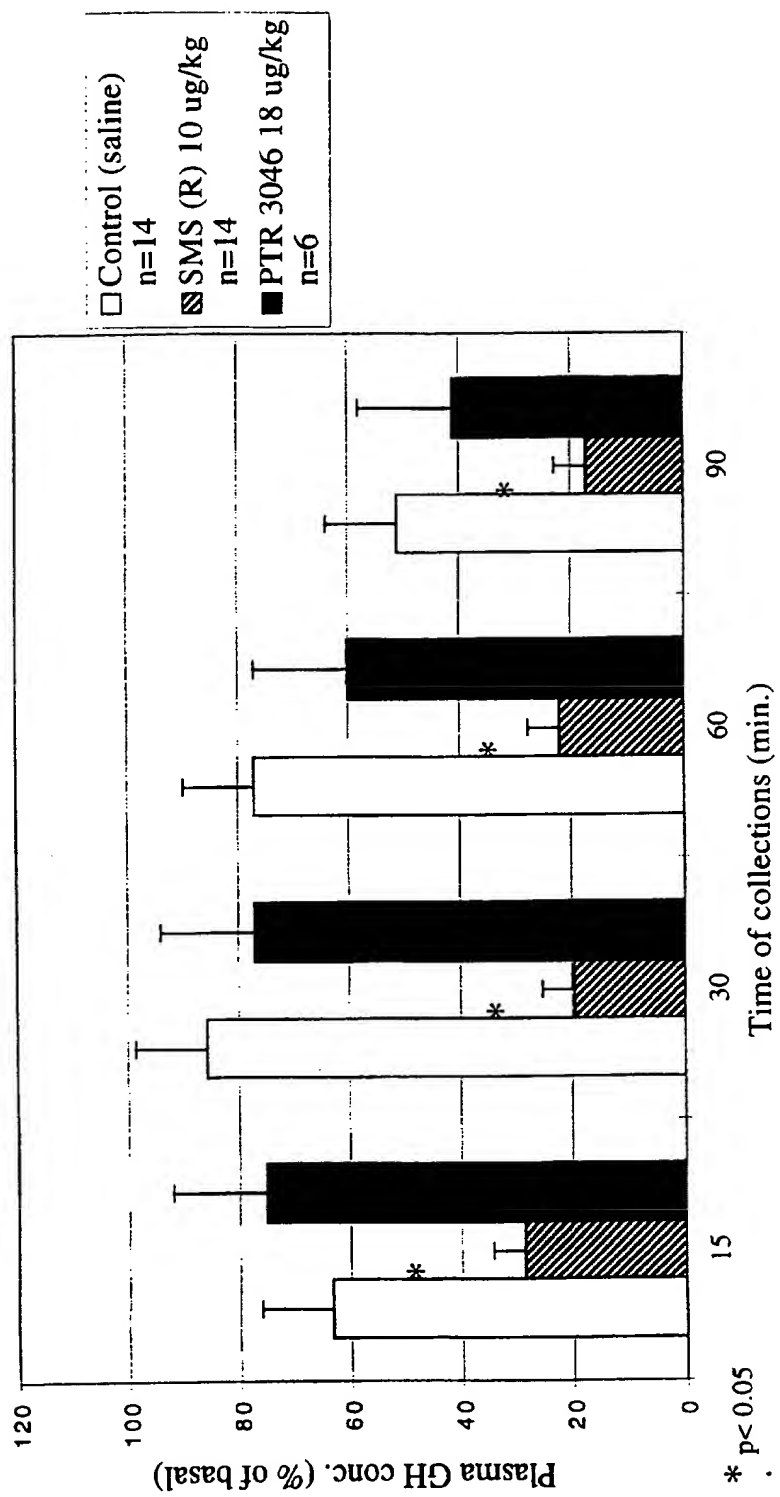Figure 2. Inhibition of ^{125}I-SRIF binding to AtT20 cells
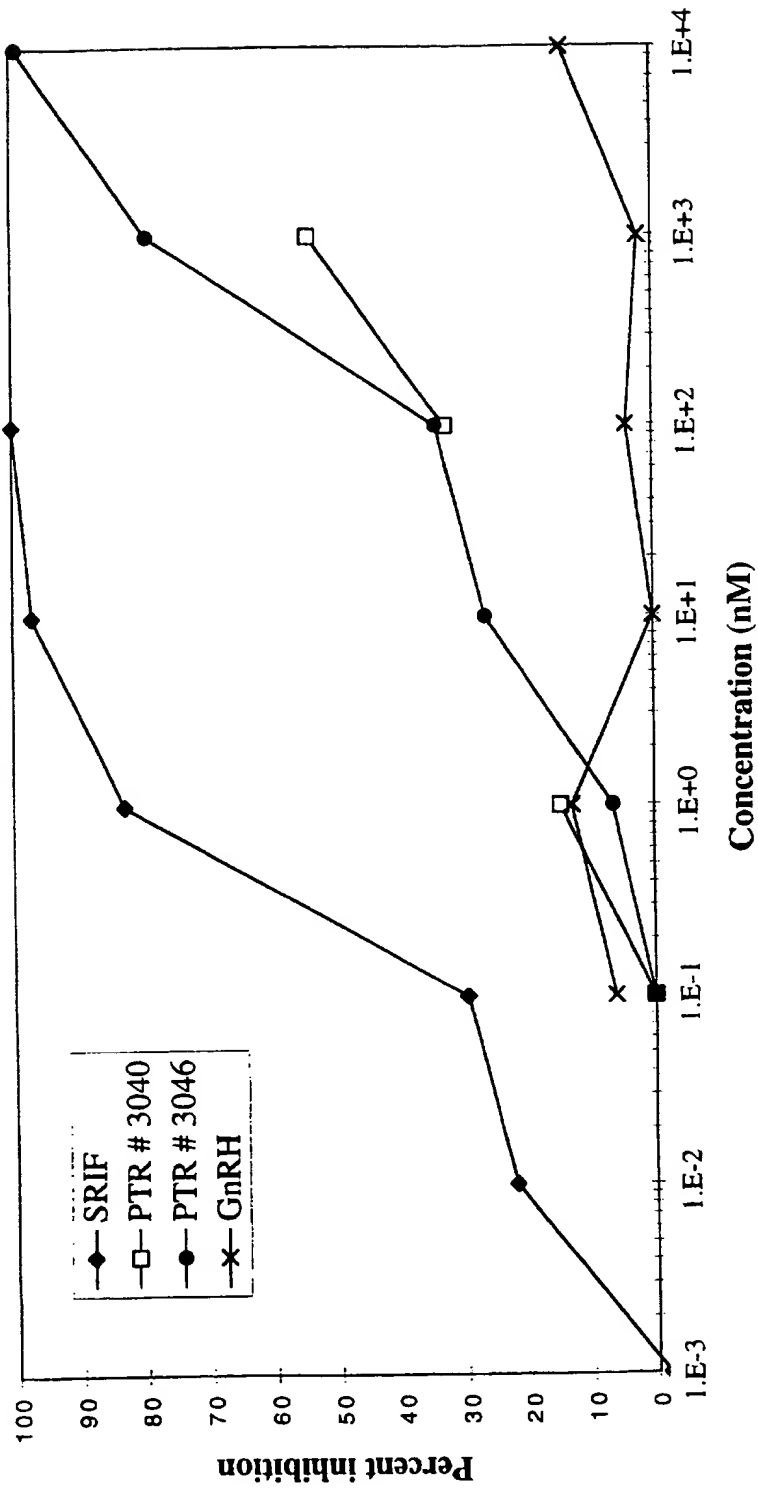
Figure 3. The *in vivo* effect of SST analogs on GH release in rats

Figure 4. The in vivo effect of SST analogs on glucose induced insulin release in rats
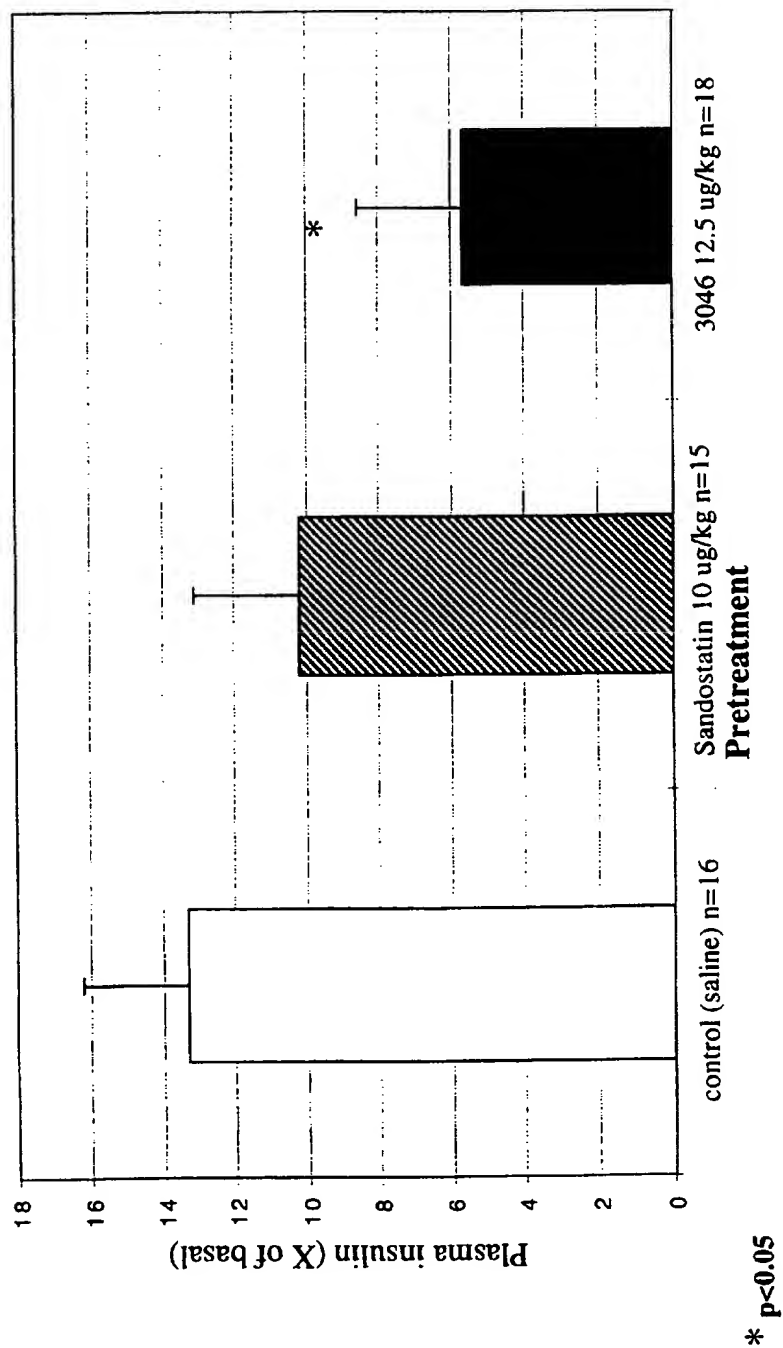
Figure 5. Pancreatic exocrine release in duodenal perfusion following bombesin induction
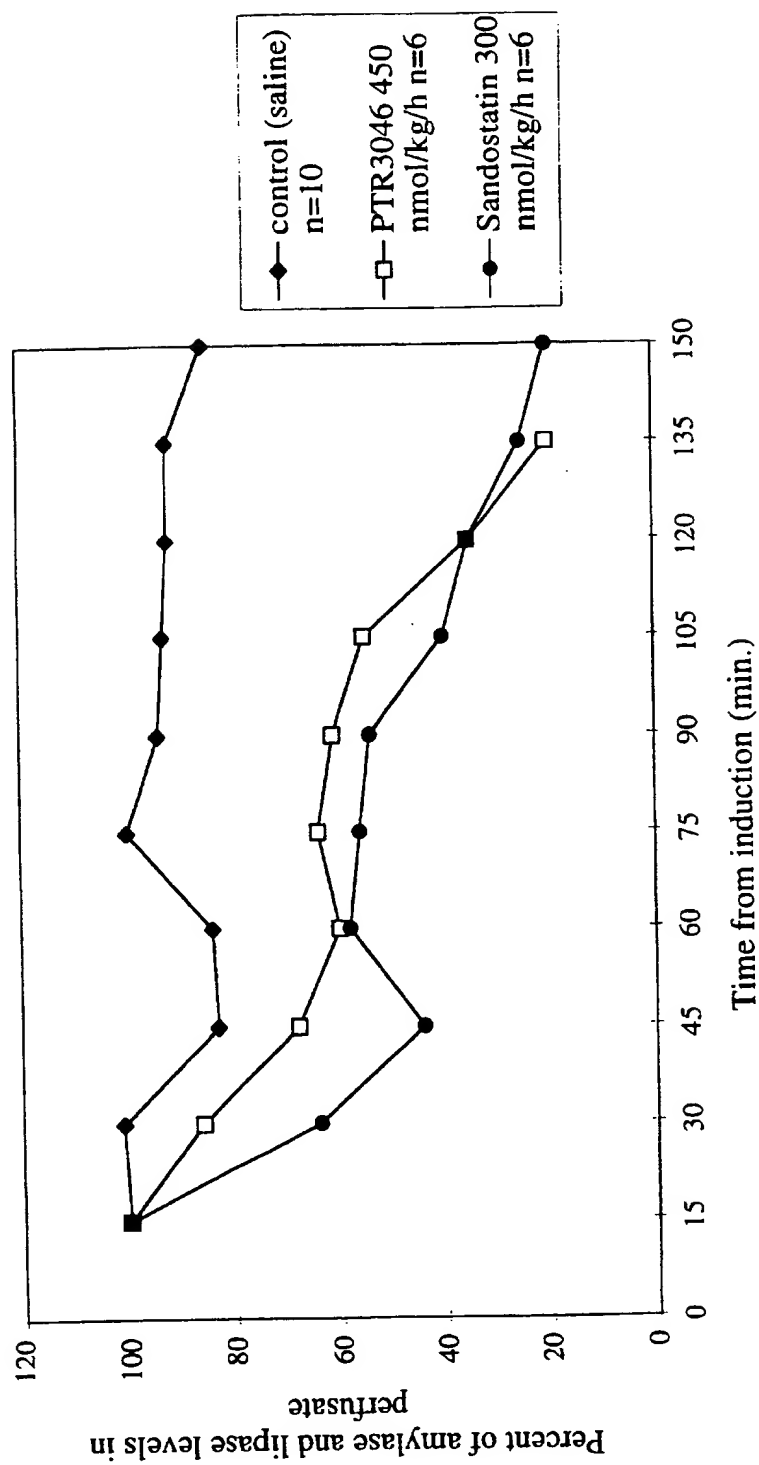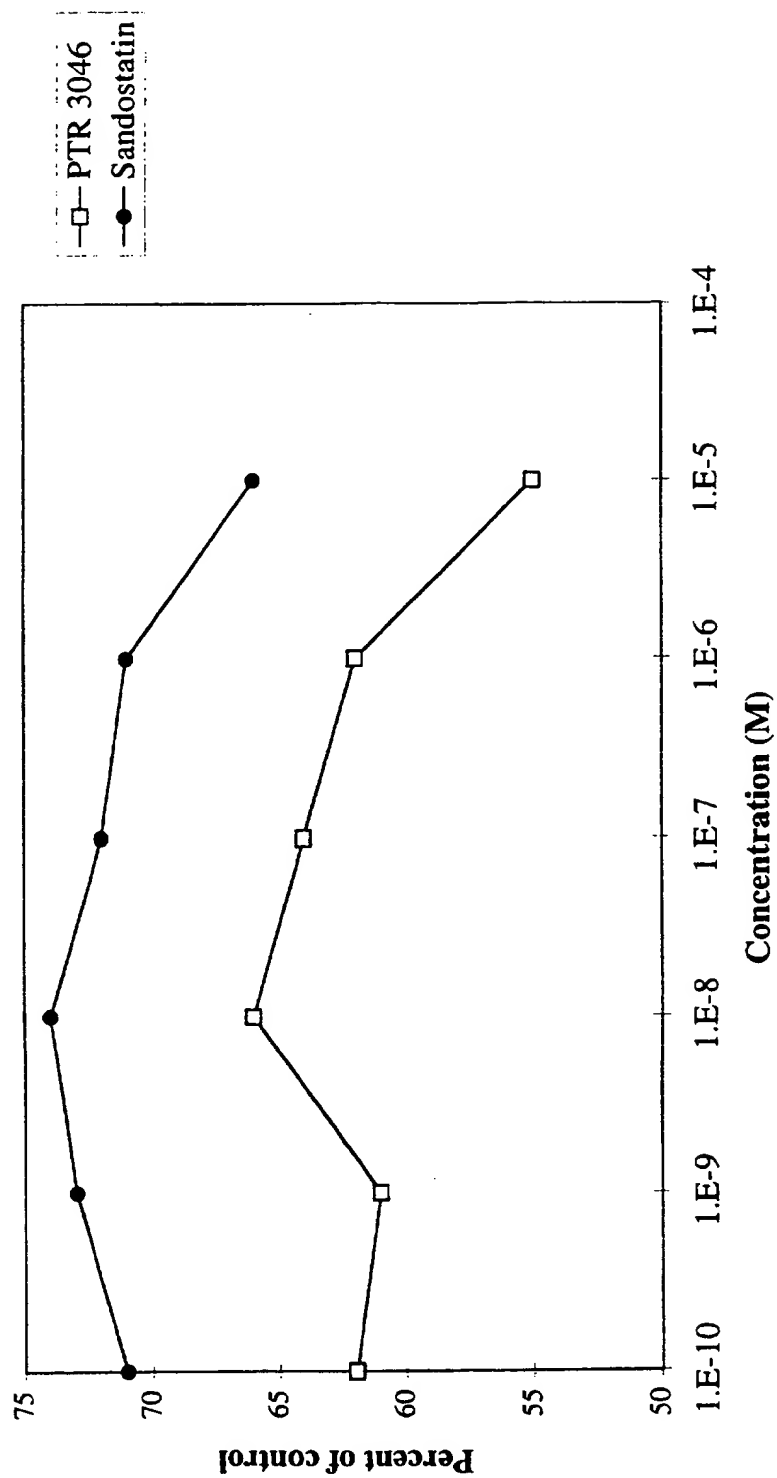
Figure 6. In-vitro antiproliferative effect of PTR3046 on MiaPaca-2 cells

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :C07K 7/50; A61K 38/04, 38/12
US CL   :530/311, 317, 328; 514/11, 16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   530/311, 317, 328; 514/11, 16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN

search terms:somatostatin, backbone cyclization, monocylclic

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y — A	EP 0 564 739 A2 (YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM) 13 October 1993 (13.10.93), see entire document.	1-18 ---- 1-18
Y	GILON et al. Backbone Cyclization: A New Method For Conferring Conformational Constraint on Peptides. Biopolymers. 1991, Vol. 31, pages 745-750, see entire document.	1-11
A	BYK et al. Building Units for N-Backbone Cyclic Peptides. 1. Synthesis of Protected N-(ω-Aminoalkylene)amino Acids and Their Incorporation into Dipeptide Units. Journal of Organic Chemistry. 1992, Vol. 57, No. 21, pages 5687-5692.	1-11

[X] Further documents are listed in the continuation of Box C.   [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 SEPTEMBER 1997	1 2 DEC 1997

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	ANISH GUPTA
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y -- A	EP 0 395 417 B1 (THE ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND) 08 February 1995 (08.02.95), see entire document.	1-18 ---- 1-18
A	US 4,011,182 (SARANTAKIS) 08 March 1977, see entire document.	1-18
A	RUDINGER, J. 'Characteristics of the amino acids as components of a peptide hormone sequence' In: Peptide Hormones. Edited by J.A.Parsons. Baltimore: University Park Press, 1976. pages 1-7.	1-18